View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

International Journal of Mycobacteriology 3 (2014) 88–93

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/IJMYCO

Evaluation of three immunological tests for the diagnosis of pulmonary tuberculosis in a rural endemic area of Bangladesh

Mohammad Tariqul Islam [a], *Fazle Rabbi* [b], *Shameema Ferdous* [c], *Ummey Shahnaz Parvin* [d], *Akram Hossain* [a], *Mohammad Sorowar Hossain* [b,*]

[a] *Department of Microbiology, Mymensingh Medical College, Mymensingh, Bangladesh*
[b] *Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University, Dhaka, Bangladesh*
[c] *Department of Microbiology, University of Dhaka, Dhaka, Bangladesh*
[d] *Department of Microbiology, East West Medical College, Dhaka, Bangladesh*

ARTICLE INFO

Article history:
Received 11 March 2014
Accepted 17 March 2014
Available online 2 May 2014

Keywords:
Pulmonary tuberculosis
Immunological tests
Bangladesh

ABSTRACT

Objective: Bangladesh is a high tuberculosis burden country. It is always challenging to diagnose active pulmonary tuberculosis (PTB) cases in rural areas where the setting up of conventional microscopic and cultural diagnostic tools is difficult. The objective of the present study is to find a feasible, reliable and easily accessible alternative diagnostic approach for PTB in the rural areas of Bangladesh.

Methods: A total of 86 sputum samples were collected from clinically suspected PTB patients of Anantapur village, an underdeveloped remote area of Netrokona district, Bangladesh. Sputum samples were screened by Ziehl–Neelsen (Z–N) and fluorescence staining methods and were categorized as smear-positive active PTB cases ($n = 50$) and smear-negative controls ($n = 36$); then the performance of three popular immunological tests were evaluated, including ICT, ELISA and Mantoux tests (MT).

Results: The sensitivity of ICT, ELISA, and MT (10 mm induration size) was 68%, 84% and 96%, respectively, and the specificity of these tests was 94.4%, 80.6% and 52.8%, respectively. When the cut-off size of induration in MT was changed from 10 to $\geqslant$15 mm, the sensitivity and specificity of MT became 92% and 83.3%, respectively. It was also found that the interpretation of MT was not significantly affected by BCG vaccination when $\geqslant$15 mm induration was taken as a cut-off value.

Conclusion: Considering the resource-constraints of rural and remote areas, the Mantoux test could be an alternative tool for the diagnosis of active PTB.

© 2014 Published by Elsevier Ltd. on behalf of Asian-African Society for Mycobacteriology.

* Corresponding author. Address: Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University, 66 Mohakhali, Dhaka 1212, Bangladesh. Tel.: +880 2 8824051 4x4080; fax: +880 2 8810383.

E-mail addresses: ronyyoga@gmail.com (M.T. Islam), fazle@bracu.ac.bd (F. Rabbi), shameemaferdous@yahoo.com (S. Ferdous), rupammc@gmail.com (U.S. Parvin), akram.prof@gmail.com (A. Hossain), sorowar.hossain@bracu.ac.bd (M.S. Hossain).

http://dx.doi.org/10.1016/j.ijmyco.2014.03.003
2212-5531/© 2014 Published by Elsevier Ltd. on behalf of Asian-African Society for Mycobacteriology.

Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* is a major public health concern in Bangladesh and causes more deaths than any other infectious diseases [1]. Pulmonary tuberculosis (PTB) is the most common form of TB worldwide. According to the World Health Organization (WHO), Bangladesh ranks sixth among the world's 22 high-burden TB countries. The TB mortality rate (45 deaths per 100,000 populations) is the highest in Bangladesh than any other Southeast Asian countries (31 deaths per 100,000 populations) [2,3].

Early diagnosis followed by proper medications is critical for the prevention of TB-associated morbidity and mortality. Acid-fast bacilli (AFB) microscopy is traditionally used as the first diagnostic test for screening active TB disease in many areas of Bangladesh where the access to a culture facility is limited [4]. For developing countries with a large number of cases and financial constraints, demonstration of acid-fast-bacilli in smears has great importance [5–8]. Direct smear is a relatively fast and cheap method, but it lacks sensitivity (40–60%), since it relies substantially on the quality of sputum samples and requires technical expertise, which is usually unavailable in poor rural areas [9]. In this context, a number of alternative diagnostic tests have been developed using molecular, chromatographic and immunological approaches. Immunological tests make use of specific humoral or cellular responses of the host [10]. Immunological tests have several advantages over other diagnostic methods because of their speed, technological simplicity and modest training requirements. In addition, these tests could be performed at peripheral health facilities without onsite microscopic services. These tests are available in different formats such as enzyme-linked immunosorbent assay (ELISA), immunochromatographic test (ICT) and Mantoux test (MT). As expected, ELISA holds great promise in the diagnosis of active TB [11] due to its sensitivity and reproducibility. ICT employs strip tests, which are ideal for home testing, rapid point care of testing, and testing in the field. It does not require any special equipment or technical skill [12,13]. The century old Mantoux test detects cutaneous sensitivity to purified protein derivatives which develop on exposure to *M. tuberculosis* and other related mycobacteria. MT is relatively cheap and can be carried out easily by general health workers in resource-constrained remote endemic areas. But the main limitation of MT is BCG vaccination might influence the final outcome due to false positivity. Nonetheless, it could be an important tool for diagnosis of both active and latent TB infections in TB endemic areas [14]. According to a recent survey, immunological tests are widely used in high-burden countries including Bangladesh and her neighboring countries like India, Pakistan, Myanmar, Thailand and China [15]. However, there are no published reports on the comparative performance of different immunodiagnostic tests available in Bangladesh. Therefore, in the present study, the aim was to compare the performances of three commonly used immunological tests in diagnosing active PTB to find out a feasible, reliable and easily accessible diagnostic approach for rural areas of Bangladesh.

Methods

Study area and study participants

This study was conducted in a resource-limited rural setting of Anantapur village from January 2008 to December 2008. Anantapur is an underdeveloped remote area of Netrokona district (200 km north-west of Dhaka city), Bangladesh. More than 90% of the study participants came from a low-income family with an average monthly income of about $25–$40 (about 2000–3000 Taka). The Majority of them were smokers. PTB suspected patients were recruited at the TB field clinic run by Demien Foundation-Bangladesh, Netrokona; 58 suspected patients presenting with clinical features suggestive of TB were initially screened at the clinic. Healthy volunteers ($n = 28$) from Anantapur village and clinic staff were enrolled as healthy controls. The health status of the control participants was examined by the study clinician. Both cases and controls belonged to same age group with a median age of 41 years. All study participants including suspected and healthy individuals were screened by sputum smear microscopic examination.

Specimens

Morning sputum samples were collected for smear examination. The sputum samples were digested and decontaminated with 2% sodium hydroxide and then processed for further investigation. Ziehl-Neelson (Z–N) acid fast staining and auramine O fluorochrome staining were used to confirm the presence of tubercle bacilli. Venous blood was collected from all the patients and control subjects. Blood was allowed to clot, and after centrifugation at 1500 rpm for 10 min, the serum was separated and stored at −20 °C until it was used.

Microscopic examination of sputum smears

Z–N and fluorochrome staining procedures were used for microscopic sputum smear examination. Both staining protocols and microscopic reporting were done according to WHO laboratory guidelines [16]. Heat fixed sputum smear was stained using the Z–N method with carbolfuchsin. After rinsing with water and decolorizing using 3% acid-alcohol, the smear was stained with methylene blue. When the smear was examined under light microscope in ×100 oil immersion, tubercle bacilli appeared as fine red rods, slightly curved, more or less granular, isolated, in pairs or in groups against the blue background. For fluorochrome staining, the smear was first flooded with fluorochrome stain auramine O. After rinsing with water and decolorizing using 0.5% acid-alcohol, the smear was counterstained by ink blue or pelican 4001. Finally, the smear was air dried and examined under a fluorescent microscope. The tubercle bacilli appeared as yellow luminous rods against a dark background.

ELISA

A commercially available ELISA kit (Anda Biologics, France) that detects the IgG antibody to the A-60 antigen of *M.*

tuberculosis was used in this study. The test was carried out according to the manufacturer's protocol. First, the serum samples were diluted 1:100 in the sample diluents and 100 ml of the diluted samples and controls were added to the wells and incubated at 37 °C for 1 h. Then the wells were washed with a buffer solution and 100 μl of anti-human IgG-POD conjugate was applied to each well and incubated for 30 min at 37 °C. After that, the wells were washed again with buffer solution and 100 ml tetramethylbenzidine substrate was added in each well. The reaction was stopped by the addition of H_2SO_4 and the absorbance was measured at 450 nm using MULTISCAN-ELISA plate reader. Each sample was tested in duplicate and mean absorbance was calculated. Using the mean absorbance (O.D.) value for each sample, the corresponding concentration of antibody was determined as per calculations given in the ELISA kit and was expressed as ELISA units/ml. Samples with O.D. values greater than 0.37 were considered positive.

ICT

This test was carried out according to the manufacturer's instructions. The ICT tuberculosis diagnostic kit (CTK biotech Inc, USA) is a sandwich lateral flow chromatographic immunoassay for the detection of serum IgG and IgM antibodies to *M. tuberculosis*. Briefly, highly purified antigens secreted by *M. tuberculosis* during active infection are immobilized in test lines on the test strip. When serum is applied, it flows past the antigen lines and the antibody is detected by anti-human IgM and IgG conjugated to colloidal gold particles which produce one or more pink lines when bound to a human antibody. The presence of one or more pink lines on the test strips is considered a positive result.

MT

The test involved the introduction of tuberculin purified protein derivative (PPD) manufactured by Beacon Diagnostic Pvt, India. Ten tuberculin units (usually 0.1 ml) were injected into the upper third of the left forearm intradermally, and after three days the diameter of the induration was measured. Any palpable induration measuring 10 mm or more was considered a positive result and less than 5 mm was considered a negative result.

Statistical analysis

All the data were recorded and analyzed statistically by SPSS version 17 statistical software.

Ethical considerations

Informed consent was obtained from each participant. This study was approved by the Ethical Review Committee of Bangladesh Medical Research Council, Dhaka (ref: BMRC/ERC/2007-2010/875).

Results

Diagnosis of microscopically confirmed PTB cases

A total of 86 sputum samples were screened by Z–N and fluorescence staining methods. Among the study participants, 50 patients were finally classified clinically and microscopically (smear positive) as active PTB cases. On the other hand, 36 samples were smear negative, which were categorized as controls. Smear-negative controls included 13 contacts with TB patients in the family and TB clinics, 8 patients with non-tubercular lung diseases and 15 healthy volunteers.

Comparative performance of ICT, ELISA and MT for the diagnosis of active PTB cases

The sensitivity, specificity, positive prediction values and negative prediction values of ICT, ELISA and MT are shown in Table 1. The sensitivity of the assays ranged from 68% to 96%, with the lowest being for ICT (68%) and the highest being for MT with 10 mm induration as cut-off size (96%). On the other hand, the specificity of the assays ranged from 52.8% to 94.4%, with the lowest being for MT with 10 mm induration as cut off size (52.8%) and the highest being for ICT (94.4%). It was also found that when the cut-off value of MT was changed from 10 to $\geqslant$15 mm induration size, the sensitivity slightly deceased from 96% to 92%, but the specificity increased sharply from 52.8% to 83.3%. Positive predictive values for ICT, ELISA, MT of 10 mm, and MT $\geqslant$ 15 mm cut-off values were 94.4%, 85.7%, 73.8% and 88.5%, respectively, while the negative predictive values were 68%, 78.4%, 90.5% and 88.2%, respectively (Table 1). In addition, mean values of indurations in MT for both case and control groups were measured and the pulmonary TB cases and healthy volunteers showed the highest (23.0 mm SD ± 6.4) and lowest (7.0 mm SD ± 2.5) mean indurations, respectively (Table 2).

Effect of BCG vaccination on MT interpretation

The effect of BCG vaccination in the interpretation of MT was studied by taking mean MT induration values for BCG vaccinated and no-vaccinated groups. The mean induration value for the vaccinated group was 11.4 mm (SD ± 4.54), while the mean induration value was 7.2 mm (SD ± 3.0) for the non-vaccinated group (Table 3). When the difference between the mean MT indurations between the case and control groups was calculated, it was highly significant by independent sample *t*-test ($P < 0.001$).

Discussion

The rapid and accurate diagnosis of infected individuals is the basis of any TB control program. The sensitivity and specificity of immunological tests tend to show variation in different demographic characteristics or geographical background due to different levels of immunological responses [9,17]. This study, conducted in a homogeneous population of both microscopically confirmed cases and non-TB controls, showed that MT is better in terms of sensitivity and specificity

Table 1 – Comparison of immunological tests for the diagnosis of TB.

Tests	Results	PTB cases (total 50 individuals)	Controls (total 36 individuals)	Sensitivity (%)	Specificity (%)	Positive prediction value (%)	Negative prediction value (%)
ICT	Positive	34	2	68	94.4	94.4	68
	Negative	16	34				
ELISA	Positive	42	7	84	80.6	85.7	78.4
	Negative	8	29				
MT (⩾10 mm cut-off)	Positive	48	17	96	52.8	73.8	90.5
	Negative	2	19				
MT (⩾15 mm cut-off)	Positive	46	6	92	83.3	88.5	88.2
	Negative	4	30				

Table 2 – Mean values of indurations in MT for case and control groups.

Groups	Mean values of MT indurations (standard deviation)
PTB cases	23.0 mm (±6.4)
Non TB lung patients	10.0 mm (±3.0)
Contact persons	12.6 mm (±4.9)
Healthy volunteers	7.0 mm (±2.5)

Table 3 – Effect of BCG vaccination on mean values of MT indurations for control group.

Groups	No. of individuals	Mean values of MT indurations (standard deviation)
Vaccinated[a]	20	11.4 mm (±4.5)
Non-vaccinated[a]	16	7.6 mm (±3.0)

a Independent *t*-test is highly significant between two groups ($P < 0.001$).

as compared with those of ELISA and ICT for TB diagnosis in a rural and remote endemic area of Bangladesh.

In the present study, the sensitivity and specificity of ICT were found to be 68% and 94.4%, respectively (Table 1). These findings are in agreement with the Italian study where the sensitivity and specificity were 66.7% and 90.4%, respectively [12]. Another study carried out in India showed similar sensitivity (70.8%) and specificity (92.2%) [18]. In contrast, poor sensitivity (40%) of ICT was observed in Turkey, but the specificity was found to be 100% [19]. In the case of ELISA, the sensitivity and specificity were 84% and 80.6%, respectively (Table 1). A similar pattern of sensitivity (80.77%) and specificity (88.4%) was also observed in Taiwanese people [20]. A similar study carried out in Pakistan showed that the sensitivity (88.8%) was higher, but the specificity (69.8%) was much lower as compared with this study [21]. The variations in performances of ICT and ELISA might be due to different levels of antibody responses towards tuberculosis infection at various stages of disease and also the heterogeneity of the study population in different parts of the world [9,17].

MT is very much underutilized for tuberculosis control in developing countries, including Africa and Asia. A major argument against using the tuberculin skin test for diagnosis of active TB is that prior BCG vaccination usually interferes with the interpretation of a positive skin test [22]. In this study, the MT test was found to be a more sensitive tool than ICT or ELISA with 96% sensitivity, but its specificity was low (52.77%) when 10 mm induration size was taken as a cut-off value. However, its sensitivity and specificity changed to 92% and 83.3%, respectively, when the induration diameter of ⩾15 mm was taken as a cut-off size. Considering this cut-off value (⩾15 mm,) the performance of MT was better than any other serological test evaluated in the present study (Table 1). Similar results were observed in a previous study [17] where the specificity of MT improved, but sensitivity was unchanged when the cut-off value was considered as ⩾15 mm. The diagnostic potential of a test in clinical practice also depends on its predictive values. High positive predictive values of a test make the test useful in strengthening the clinical suspicion of disease, while high negative predictive values of the test makes the test useful in the exclusion of disease in negative cases. MT showed a higher positive predictive value of 88.5% and a negative predictive value of 88.2% at ⩾15 mm cut-off value. In this study, MT with an induration size of ⩾15 mm was found to be effective in the screening of PTB cases (Table 2), and the *t*-test result showed that the interpretation of the MT induration results was not interfered with by the BCG vaccination during the diagnosis of PTB cases when ⩾15 mm was considered as the cut-off value (Table 3).

The effect of vaccination on MT (PPD skin test) has been observed to decline over time, with less than 10% of recipients retaining tuberculin positivity 10–15 years after vaccination [23,24]. A meta-analysis of 26 studies has strongly suggested that MT indurations of >15 mm is more likely to be caused by active TB infection than the effect of previous BCG vaccination [24]. Therefore, the widespread BCG vaccination in childhood in this population should not be considered as a limiting factor to use MT as a tool for diagnosis of TB in adult [23]. The overall health service system in low-income countries is in a dire state due to lack of basic infrastructure and health professionals, and running short of supplies and drugs. Bangladesh is a low-income country where over 70% of the

population lives in rural areas. Approximately 31.5% of the population lives below the poverty line, and 26% of the population are undernourished [25,26]. Bangladesh is well known for its effective TB program in rural areas spearheaded by non-governmental organizations. Nonetheless, it substantially fails to reach the marginalized population in rural and remote areas where the prevalence of TB is the highest [27]. As discussed earlier, though sputum smear microscopy is still one of the most widely used diagnostic tools for the screening of tuberculosis in high TB burden developing countries, it lacks sensitivities and also requires laboratory skills.

Considering the resource-constrained rural and remote areas of Bangladesh, the Mantoux test could be a promising alternative tool for the diagnosis of active PTB and could assist in the disease prognosis because it is relatively cost-effective and can be carried out even without primary laboratory setup and a technical person as compared with other diagnostic tests, including microscopic, cultural and immunological approaches. The small sample size is the major limitation of this study, which was conducted in a small rural and remote Anantapur village of Bangladesh. Further evaluation is required in other rural areas with a larger sample size to establish the Mantoux test as an alternative diagnostic test for active PTB.

Disclosure

We declare that we do not have any conflict of interest.

Acknowledgements

This work was a part of MPhil thesis project and was conducted without external financial support. The authors would like to thank Demien Foundation-Bangaladesh, Netrakona, for its support.

REFERENCES

[1] D.R. Guda, I.U. Khandake, S.D. Parveen, T. Whitson, Bangladesh: NGO and Public Sector Tuberculosis Service Delivery-Rapid Assessment Results, University Research Co, LLC, Bethesda, MD, 2004.
[2] World Health Organization. Global Tuberculosis Report 2012. Available from: <http://www.who.int/tb/publications/global_report/gtbr12_main.pdf>, 2013 (24.11.13).
[3] National guidelines and operational manual for tuberculosis control, fourth ed., DGHS, WHO country office for Bangladesh; 2009, pp. 1–14.
[4] Diagnostic standards and classification of tuberculosis in adults and children. This official statement of the American Thoracic Society and the Centers for Disease Control and Prevention was adopted by the ATS Board of Directors, July 1999. This statement was endorsed by the Council of the Infectious Disease Society of America, September 1999. Am. Respir. Crit. Care Med. (161) (2000) 1376–1395.
[5] K.R. Steingart, N.V. Vivienne, M. Henry, Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review, Lancet Infect. Dis. 6 (2006) 664–674.
[6] B.A. Forbes, D.F. Sahm, A.S. Weissfeld. Role of microscopy in the diagnosis of infectious diseases, in: Bailey, Scotts (Eds.), Diagnostic Microbiology, tenth ed., Mosby Co, Missouri, 1998, pp. 134–149.
[7] A. Jain, A. Bhargava, S.K. Agrawal, A comparative study of two commonly used staining techniques for acid fast bacilli in clinical specimens, Indian J. Tuberc. 2002 (49) (2002) 161–162.
[8] A. Somoskovi, J.E. Hotaling, M. Fitzgerald, D. Donnel, L.M. Parsons, M. Salfinger, Lessons from a proficiency testing event for acid-fast microscopy, Chest 120 (2001) 250–257.
[9] S. Suraiya, M. Musa, R. Suppian, J.A. Haq, Serological diagnosis for active tuberculosis in Malaysian population: comparison of four protein candidate, Asia Pac. J. Trop. Dis. 2 (2012) S312–315.
[10] S. Pottumarthy, V.C. Wells, A.J. Morris, A comparison of seven tests for serological diagnosis of tuberculosis, J. Clin. Microbiol. 38 (2000) 2227–2231.
[11] Y. Kalantri, N. Hemvani, G.C. Bhatia, D.S. Chitnis, Elisa kit evaluation for IGG and IGM antibodies to A-60 tubercular protein antigen, Indian J. Med. Sci. 59 (2005) 337–346.
[12] A. Bartolini, M. Strohmeyer, F. Bartalesi, D. Messeri, E. Tortoli, A. Farese, et al, Evaluation of rapid immunochromatographic test for the serological diagnosis of tuberculosis in Italy, Clin. Microbiol. Infect. 9 (2003) 632–639.
[13] M. Singh, C. Espitia, Immunological diagnosis in tuberculosis, in: J.C. Palomino, S. Leao, V. Ritacco (Eds.), Tuberculosis, first ed., Online Textbooks, 2007, pp. 425–431.
[14] B. Jamil, S. Qamruddin, A.R. Sarwari, R. Hasan, An assessment of Mantoux test in the diagnosis of tuberculosis in a BCG-vaccinated, tuberculosis-endemic area, Infect. Dis. J. Pak. 17 (2008) 18–22.
[15] K.R. Steingart, A. Ramsay, D.W. Dowdy, M. Pai, Serological tests for the diagnosis of active tuberculosis: relevance for India, Indian J. Med. Res. 135 (2012) 695–702.
[16] I.N. de Kantor, S.J. Kim, T. Frieden, A. Laszlo, F. Luelmo, P.Y. Norval, et al, Laboratory Services in Tuberculosis Control Part II: Microscopy, World Health Organization, Geneva, 1998.
[17] S. Singh, J. Singh, S. Kumar, K. Gopinath, V. Balooni, N. Singh, et al, Poor performance of serological tests in the diagnosis of pulmonary tuberculosis: evidence from a contact tracing field study, PLoS One 7 (2012) e40213.
[18] D.G. Tripathi, N. Sriram, V.K. Naik, P. Smita, A. Seema, G. Shakila, et al, Efficacy of immunochromatographic technique for the sero diagnosis of tuberculosis, Indian J. Med. Microbiol. 22 (2004) 131–132.
[19] G. Ongut, D. Ogunc, F. Gunseren, C. Ogus, L. Donmez, D. Colak, et al, Evaluation of ICT tuberculosis test for the routine diagnosis of tuberculosis, BMC Infect. Dis. 6 (2006) 37.
[20] I.H. Chiang, J. Suo, K.J. Bai, T.P. Lin, K.T. Luh, C.J. Yu, et al, Serodiagnosis of tuberculosis. A study comparing three specific mycobacterial antigens, Am. J. Respir. Crit. Care Med. 156 (1997) 906–911.
[21] S.O. Ahmed, S.A. Kharal, S.S. Hasan, S.S. Shah, N. Rahat, B. Begum, Elisa method evaluation of anti A60 IgG for the diagnosis of tuberculosis, JLUMHS 7 (2008) 25–30.
[22] K. Nelson, Tuberculin testing to detect latent tuberculosis in developing countries, Epidemiology 18 (2007) 348–349.
[23] R. Menzies, B. Vissandjee, Effect of bacilli Calmette-Guérin vaccination on tuberculin reactivity, Am. Rev. Respir. Dis. 145 (1992) 621–625.
[24] L. Wang, M.O. Turner, R.K. Elwood, M. Schulzer, J.M. FitzGerald, A meta-analysis of the effect of Bacille Calmette Guérin vaccination on tuberculin skin test measurements, Thorax 57 (2002) 804–809.
[25] A. Islam, Bangladesh health system in transition: selected article, Monograph Series: 11, JPGSPH, BRAC University, 2009. Available from: <http://dspace.bracu.ac.bd/bitstream/handle/10361/617/series%2011.pdf?sequence=1>, (24.11.13).

[26] Central Intelligence Agency. The world fact book. Available from: <https://www.cia.gov/library/publications/the-world-factbook/geos/bg.html>, 2013 (24.11.2013).
[27] S. Hossain, M.A. Quaiyum, K. Zaman, S. Banu, M.A. Husain, M.A. Islam, et al, Socio economic position in TB prevalence and access to services: results from a population prevalence survey and a facility-based survey in Bangladesh, PLoS One 7 (2012) e44980.