Proceedings S.Z.P.G.M.I vol: 12(3-4) 1998, pp. 66-69.

Immunological Serodiagnosis of Pulmonary Tuberculosis in Adults Using Antigen 60

Omar Iqbal Khan, Syed Talal Arshad, Yasir Abbas Naeem, Jamila Iqbal Khan
Department of Microbiology, Shaikh Zayed Hospital, Lahore.

SUMMARY

Tuberculosis has been declared as a "global emergency" by WHO. Various conventional diagnostic techniques used for the successful isolation of mycobacteria are time consuming and low in sensitivity. Many attempts have been made to develop rapid sero-diagnostic techniques with the production of commercial kits to be used in the clinical laboratories. This study investigates the efficacy of such a test based on the antibody levels to A_{60} antigen of the mycobacterium for the diagnosis of pulmonary tuberculosis. The test kit is a manufactured product supplied by Anda Biologicals, France. The study was performed on 80 subjects including 20 controls. Taking AFB smear / culture as `gold standard' test sensitivity and specificity of the kit was calculated. Of the 30 AFB and culture positive, 24 cases were found positive with Anda giving sensitivity of 80% while out of 20 control cases which had no clinical signs / symptom or history of TB disease or exposure, 3 were positive giving a false positive percentage of 15%. Specificity was calculated as 85%. Of the 10 patients treated for TB and at the time of testing negative for AFB and culture, 90% sensitivity was reported. BCG vaccinated positive pulmonary TB cases gave 83.33% sensitivity, while BCG negative TB positive cases showed 82.3% sensitivity. BCG negative and TB negative cases gave 15.78% positive Anda test result. Male and female results did not show significant variation. Since all organisms isolated were mycobacterium tuberculosis interspecificity could not be determined with this trial.

INTRODUCTION

Mycobacterium tuberculosis produces a slow growing disease causing extensive destruction forming granulomatous lesions, with or without caseation[1]. Pulmonary involvement of the disease is the most common[2] but the organisms may disseminate from the lungs to any part of the body. Nearly 8 million cases are reported each year with 3 million deaths worldwide[3]. Successful isolation of these bacteria depends upon collection of proper specimens and is time consuming. The growth of bacteria is slow and isolation on culture takes about 6-8 weeks[4], before which the affected person has to be started on a drug regime. Acid Fast Bacillus (AFB) is recovered about 30% to 50% of the clinical cases and culture is positive in only 50% of these cases[5]. Microscopic examination undertaken by most of the clinical laboratories, has the advantage of getting the result on the same day but it is likely to be positive only if large number of bacilli are present i.e. 100,000/ml by Ziehl-Neelsen technique of 10,000/ml in flourochrome staining[6,7].

Rapid diagnostic methods like measuring the evolution of $^{14}CO_2$ from radiolabelled substrate in the medium[8] and DNA probing for the direct detection of specific mycobacterial DNA lack sensitivity[9]. Gas chromatography and mass spectroscopy although highly sensitive and specific[10], is not widely accepted because workers doing such tests are not convinced of this being a cost effective method for diagnosis of TB. DNA amplification by polymerase chain reaction (PCR) and related methods are more sensitive and are availabe commercially at few places but suffer from complexity of technique and interpretation of the results to the physicians[11]. These problems led to many attempts to develop serodiagnostic tests for

tuberculosis[12,13]. Despite huge amounts of efforts no serodiagnostic tests is widely used in the clinical laboratories because the overlap between levels of antibodies present in healthy individuals exposed or treated and patients suffering from disease, is unacceptably large. Natural antibodies may be produced due to contact or exposure with mycobacterium and related genera in the environment.

Tuberculin test or Mantoux test frequently requested by the physicians, is usually not done with standard dose of tuberculin which makes its interpretation difficult particularly amongst the population of high prevalence of disease[14]. The present epidemiological situation of tuberculosis[15,16] has intensified the need for accurate and preferably simple, rapid and cost-effective test. This study investigates the efficacy of one such test based on the antibody levels to A60 antigen of mycobacterium. The test kit is a manufactured product supplied by Anda Biologicals, France.

MATERIALS AND METHODS

Testing was performed as directed. Fifty microlitre of whole blood or 25 microlitre of serum/plasma was deposited in antigen A60 (derived from M.bovis) coated wells of the microtiter plates (plastic trays manufactured by Anda Biologicals , Strasbury France). 200 microliter of activator/dilutent was added and the plates were left horizontally at a flat surface and the results was read after 15 minutes. According to the manufacturer, a strong black line/purple line indicates successful employment of the test while a black-red line in the test window indicates antibodies (IgG, IgM, and/or IgA) present against mycobacterium A 60, meaning the patient is seropositive. No line in the test zone means the patient is sero-negative. The proposed sensitivity of the tests stands at 400 arbitrary units for both IgA and IgG and a concentration of IgM antibodies corresponding to an adsorbance unit in the regular Anda ELISA dilution of serum 1:100. The test is proposed to be inter-specific. Mycobacterium was isolated by standard conventional culture techniques with Lowenstein-Jensen, Middlebrook 7H10 media[17]. Eighty cases within five groups were selected for testing.

Group I: comprising of 20 cases was of diagnosed pulmonary tuberculosis both on clinical signs and symptoms as well as laboratory results. Group II: comprised of TB cases treated with Anti Tuberculous Treatment (ATT) for at least six months and was now Acid Fast Bacilli negative as well as culture negative. Ten cases were selected in this group. Group III was of ten ATT resistant cases. In Group IV 20 subjects were selected who were at a high-risk exposure of pulmonary tuberculosis and included hospital staff (doctors, nurses, paramedics etc.) and family members of group I or II. Twenty subjects who on all criteria of diagnosis of pulmonary tuberculosis were negative and were assumed to be never exposed to the disease served as controls and were assigned group V. All patients were above 22 years of age.

RESULTS

Of the 30 AFB and culture positive cases, (all harbouring Mycobacterium tuberculosis) 24 were positive with Anda giving the sensitivity of 80% while out of 20 cases which were AFB negative and had no clinical signs or symptoms or history of TB disease or exposure 3 were positive giving a false positive percentage of 15% (Fig. 1, Table 1). Specificity was calculated as 85%. Of the 10 patients treated for TB and at the time of testing negative AFB and culture 90% sensitivity was reported. BCG vaccinated positive pulmonary TB cases gave 83.33% sensitivity while BCG negative TB positive cases showed 79.167% sensitivity. BCG vaccinated TB negative cases gave 15.625% positive Anda test result (Table 2). Male and female results did not show significant variation. Since all organisms were M. tuberculosis interspecificity could not be determined with this trial.

DISCUSSION

It was Arlong in 1898[18] to use the first skin test against tubercle bacilli with a cellular reaction, which had a sensitivity of 57% and a false positivity of 11%. Until recently tests based on serology were difficult to conduct due to restricted distribution of the serology testing kits. With the commercialisation of many diagnostic tools these tests are now more common. Antigen 60 (A60) is the sixtieth antigen recorded in the counterimmunoelectrophoresis pattern[19] and is present in the wall of Mycobacterium tuberculosis. A60 is proposed to be a dominant antigen during

disease process. The commercial antigen 60 used in this trial is an antigen prepared from the cytoplasm of Mycobacterium bovis, BCG strain and is interspecific[20] Harboe and co-workers showed that during human disease and during experimental infections of rabbits[21] about 90% antibodies were directed against Antigen 60.

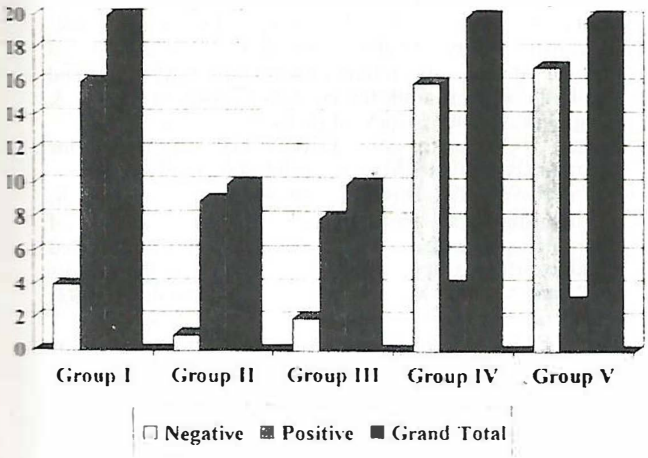

Fig. 1: **Anda serology results.**

Table 1: Anda versus AFB/culture.

	ABF / Culture				
	Positive Group I & III	*Negative Group II*	*Negative Group IV*	*Negative Group V*	*Total*
Anda +ve	24	9	4	3	40
Anda -ve	6	1	16	17	40
Total	30	10	20	20	80

The results of this trial show that the false positive ratio is too high to be ignored. Limitations include inability to test patients with immunosuppressive diseases especially HIV in which the disease is on the rise, and possibly in children[22]. The sensitivity results of this study is compared to that of 91.65% Gupta et al[23], 95% of Daftary et al[24], 82% of Qadri et al[2], and 78% of Vander Werf et al[25].

Table 2: Anda results in BCG vaccinated and non-vaccinated groups.

Groups	*Anda +ve*	*Anda -ve*	*Total*
BCG Positivve TB Positive	5 (83.3%)	1	6
BCG Negative TB Positive	28 (82.3%)	6	34
BCG Positive TB Negative	6 (15.78%)	32	38
BCG Negative TB Negative	1 (50%)	1	2
Total	40	40	80

It is interesting to see the effect of BCG on this test. Results of this study indicate not enough existing antibodies for positive serology with Anda as a result of BCG vaccination in childhood, which supports call of other scientists to abandon this vaccine as a preventive measure against pulmonary tuberculosis[26]. Yet another argument which could be given to support BCG is the patient population selected for this test; as is believed that in a country like Pakistan where the disease is so prevalent that it is publicised as the national disease, to assume that anyone with no TB exposure actually exists may be wrong. Definitely this test is another proof pointing toward the confusion due to BCG vaccination arising in sensitivity of immunology testing.

CONCLUSION

Although there is a need for improved testing methods, this test a high false positive yield. Depending on the price of this marketed product, this test may be used only as a supportive diagnostic tool in the laboratory or physician's clinic.

ACKNOWLEDGEMENTS

The authors would like to thank all physcians and staff of various teaching & out patient hospitals

and general practice clinics for helping collection of samples. We also appreciate the guidance of Dr. Majid Memon, the help of Dr. Syed Moin Ali and all the high risk indiduals of Baqai Medical University who volunteered to assist in this project.

REFERENCES

1. Lichtenberg FV. Infectious diseases. In: Robbins SL, Cotran RS (eds.) Robbins Pathologic Basis of Disease, 5th ed. WB Saunders Company, 1994; 307-380.
2. Qadri SMH, Smith. KK. Nonspecificty of the Anda A60-TB ELISA test for serodiagnosis for mycobacterium disease. Can J Micrbiol 1992; 38: 804-6.
3. Murray C, Rouillon A, Styblo K. TB, chapter 12. In: Jaamisob DT, Mosley WH, eds. Disease control priorities in developing countries population, health and nutrition division. The World Bank, June 1990; 12-13.
4. Good RC, Shinnick TM. Mycobacterium. In: Balows A, Duerden BI (eds.) Topley and Wilson's Microbiology and Medical Infections. 9th ed. Arnold, 1998; 549-70.
5. Hong Kong Chest Service / Tuberculosis Research Centre, Madras / British Medical Research Council. A study of the characteristics and course of sputum smear-negative pulmonary tuberculosis. Tubercle 1981; 62: 155-167.
6. Kim TC, Blackman RS, Heatwole KM, et al. Acid-fast bacilli in sputum smears of patients with tuberculosis. Am Rev Respir Dis 1984; 129: 264-68.
7. Maes R. Contribution to the study of the immunopathology of tuberculosis. Anda Biologicals Presentation Journal 1987; 1: 5.
8. Snider DE. Good RC, Kilburn JO, et al. Rapid drug susceptibility testing of Mycobacterium tuberculosis. Am Rev Resp Dis 1981; 123: 402-6.
9. Tenover FC. DNA hyberidization techniques and their application to the diagnosis of infectious diseases. Inf Dis Clin N Am 1993; 7: 171-81.
10. French GA, Teoh R, et al. Diagnosis of tuberculous meningitis by detection of tuberculostearic acid in cerebrospinal fluid. Lancet 1987; 2: 117-9.
11. Qureshi JA, Shah AW, Tabassum R, et al. Diseases, DNA and diagnosis: Principles of Molecular Tests in Infectios Diseases. IDJ vol. 5, Issues 2/3: 38-40.
12. Grange JM. The humoral immune response in tuberculosis: its nature biological role and diagnostic usefulness. Adv Tuberc Res 1984; 21: 71-78.
13. Wilkins EGL. The sero-diagnosis of tuberculosis. In: Clinical Tuberculosis (ed. Davis PDO), Chapman and Hall, London. 1994; p. 367-80.
14. Salahuddin N. Review oof tests for tuberculosis. IDJ 1998; 5: 41-42.
15. Kochi A. The global tuberculosis situation and the new control strategy of the WHO. Tubercle 1991; 72: 1-6.
16. World Health Organization. T.B. - A global emergency. World Health Organization, Geneva, WHO / TB 1994; 94: 177.
17. Watt B, Rayner A, Harris G. Mycobacterium. In: Collee JG, Dugoid JP, Fraser AG, Marmion BP (eds.) Mackie and McCartney, Practical Medical Microbiology. 14th ed. Churchill Livingstone, Edinburg, 1996; 329-41.
18. Arloing S. Agglutination de bacille de la tuberculose vraie. C.R. Acad. Sci. 1898; 126: 1398-1400.
19. Maes R. Anda Biologicals Presentation Journal on Contribution to the study of the Immunopathology of Tuberculosis 1987; 1: 10-16.
20. Cocito C, Baelden MC, Beboit, C. Immunological properties of antigen 60 of BCG. Scand J Immunol 1987; 25: 579-85.
21. Harrboe M, Closs O, Bjorvatn, Bjune G. Antibodies against BCG antigen 60 in mycobacterial infection. Br Med J 1977; 2: 430-33.
22. Turneer M, Van Nerom E, Nyabenda J, Waelbroeck A, Duvivier A, Toppet M. Determination of humoral immunoglobulins m and G directed against mycobacterial antigen 60 failed to diagnose primary tuberculosis and mycobacterial adenitis in children; Am J Resp & Crit Care Med 1994; 150(6 Pt 1): 1508-12.
23. Gupta S, Kumari S, Banwalikar JN, Gupta SK. Diagnostic utility of the estimation of mycobacterial Antigen A60 specific immunologlobulins IgM, IgA, and IgG in the sera of adult human tuberculosis. Tubercle & Lung Disease 1995; 76(5): 418-24.
24. Daftary VG, Banker DD, Daftary GV. Elisa Test for tuberculosis; Indian J Med Sci 1994; 48(2): 39-42.
25. Van der Werf TS, Das PK, van Soolingen D, Yong S, van der Mark TW, van der Akker R. Serodiagnosis of tuberculosis with A60 antigen enzyme-linked immunosorbent assay: failure in HIV-infected individuals in Ghana. Medical Microbiology and Immunology 1992; 181(2): 71-6.
26. Fine PEM, Rodrigues LC. Modern vaccines: Mycobacterial diseases. The Lancet; 1990; 335: 1016-20.

The Authors:

Omar Iqbal Khan
Final Year Medical Student
Baqai Medical University,
Karachi.

Syed Talal Arshad
Final Year Medical Student
Baqai Medical University,
Karachi.

Yasir Abbas Naeem
Final Year Medical Student
Baqai Medical University,
Karachi.

Jamila Iqbal Khan
Associate Professor,
Head of Microbilogy Department
Shaikh Zayed Hospital,
Lahore.

Address For Correspondance:

Dr. Jamlia Iqbal Khan
Appart. No. A/4,
Married Doctors Apptt,
Shaikh Zayed Hospital, Lahore.
e-mail- omarikhan@hotmail.com