Diagnosis Of Pulmonary Tuberculosis: Utility Of Serology And Mantoux Reaction In A Resource-Limited Setting. AUTHORS

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Citation

Abstract

BACKGROUND: The use of tuberculin skin testing in the diagnosis of tuberculosis in endemic countries is further complicated by human immunodeficiency virus infection therefore; other methods may be desirable to clarify uncertainties.
AIM: To compare Tuberculin skin testing and serology in the diagnosis of tuberculosis.METHODOLOGY: We studied 100 cases and 100 controls. Ziehl-Nelson sputum smear staining, Mantoux technique (Tuberculin Skin Test) and ELISA were used to diagnose tuberculosis. RESULTS: Fifty three percent of the cases had HIV co-infection. The Mantoux Test had a sensitivity of 45.28% in HIV/PTB patients and 21.28% in patients with PTB only. The serological test by ELISA was positive in 28/29 (96.6%) patients who had positive Mantoux test and in 21/24 (87.5%) patients with negative Mantoux. The overall sensitivity of the serologic test was found to be 94% (63/67) with specificity of 74%, while the positive and negative predictive values were 78% and 89%, respectively with efficiency of 82.5%.
CONCLUSION: Tuberculin skin test had a lower sensitivity than the serological technique in detecting cases of tuberculosis.

INTRODUCTION

The fact that Mycobacterium tuberculosis (MTB) produces a complex spectrum of immune responses is well known.[1] The tuberculin skin test is one of the methods used for identifying infection with M. tuberculosis.[2] New technology offers the opportunity for rapid alternative techniques for the diagnosis of tuberculosis and other mycobacterial diseases. Some of them may prove to be accurate, species-specific and easily applied.

Infection with MTB is invariably accompanied by hypersensitivity to tuberculin, which represents one of the first indications of contact with this organism.[3] The response in tuberculin skin testing depends on this. Upon exposure to tubercle bacilli, lymphokines are secreted from specially sensitized T-lymphocytes.[4] Humoral immune response is characterized by the production of antibodies directed against the invading agent, which forms the basis for serological tests. Studies have documented increase in total immunoglobulin levels and specific antimycobacterial antibodies in patients with TB.[5-7] Hyper-gammaglobulinaemia and raised gamma-2 globulin with a corresponding decrease in albumin have also been reported in TB cases.[8-12]

Diagnosis of tuberculosis currently depends on the demonstration of M. tuberculosis in clinical specimens. Two new approaches that may be applicable to the recognition of mycobacteria have emerged: immunoassay of mycobacterial antigens and nucleic acid probes. Immunoassay is cheap, uses stable reagents, does not require sophisticated equipment and can be performed in developing countries. This therefore, makes it potentially applicable in field settings. The utility of this has however, not been determined in our setting where Mantoux testing still remains one of the main utilized tests.

The relationship between the degree of delayed hypersensitivity reaction and the levels of antibodies to MTB, and the clinical features are far from clear.[2, 13] Although currently the tuber-culin skin test antigens that are available are less than 100% sensitive and specific for detection of infection with M. tuberculosis, no better diagnostic method is widely available.

MATERIALS AND METHODS

The study was a cross-sectional, comparative study

comprising 100 consecutive consenting adults aged 15 years and above with newly diagnosed PTB at the Medical Outpatient Department (MOPD) of the University of Maiduguri Teaching Hospital, Nigeria. The control group consisted of age- and sex-matched 100 apparently healthy, HIV-negative and PTB negative adults drawn from among hospital staff and medical students. In addition to HIV testing, all study subjects had both tuberculin skin testing (TST) employing the Mantoux (Cadila Healthcare Limited, Ahmedabad - 382445, Gujarat India) technique[14] and Enzyme-linked immunosorbent assay (ELISA) (Omega Diagnostics Limited Scotland [UK]), following the manufacturer's guidelines.

Diagnosis of Pulmonary tuberculosis (PTB) was based on the detection of Acid- and Alcohol-Fast Bacilli (AAFB) in the sputa of patients. Study subjects were clinically evaluated and blood was drawn for complete blood count (CBC), erythrocyte sedimentation rate (ESR), and serologic test for TB using ELISA. The Mantoux test reagent (PPD RT Tween 80) was injected in the volar aspect of the left arm and the reaction read after 72 hrs using a plastic rule employing the "ball point technique" of Sokal.[15] Excluded in this study were those with suspicion of extrapulmonary TB because of the challenges with diagnosis. Patients with other causes of immunosuppression e.g. chronic renal failure, diabetes mellitus, malignancies, prolonged steroid therapy were also excluded.

Generated data was analyzed using SPSS Version 11 (SPSS, Chicago, Ill, USA). Chi-square was used for categorical data in comparing the results and student t-test was used to compare means. Level of significance was pegged at p-value ≤0.05. Sensitivities, specificities, positive and negative predictive values were calculated using the method of Galen and Gambino. [16]

RESULTS

The age range was 15-56 years for cases and 14-60 years for the controls with a mean of 33.39 ±10.0 and 33.77 ± 10.56, respectively (p<0.05). Fifty three percent of the PTB patients were also HIV-infected. Tables 1 and 2 show the results of TST for patients with HIV-associated, and Pulmonary TB only, respectively. The TST was found to be positive in 55% and 79% of HIV/PTB and PTB only groups, respectively. A false positive TST was present in 12% of the control group. The specificity was 12% in both HIV/PTB and PTB only groups. The positive- and negative- predictive values were 21.4% and 10.2% in HIV/PTB and 71% and 88% in PTB

only groups, respectively.

Figure 1

Table 1. Mantoux reaction in patients with HIV-associated PTB

Reaction (mm)	Cases (n=100)	Controls (n=100)	Total
0-4	24	88	112
≥5	29	12	41
Total	53	100	153

Sensitivity: 24/53x100= 45.28%

Specificity: 12/100x100=12%

Positive predictive value: 24/112x100=21.43%

Negative predictive value: 12/41x100=29.3%

Figure 2

Table 2. Mantoux reaction in patients with PTB only

Reaction (mm)	Cases (n=100)	Controls (n=100)	Total
0-9	10	88	98
≥10	37	12	49
Total	47	100	147

Legend:
  Cases: 0-4mm - Negative
    5-9mm - Negative (PTB only)
      - Positive (HIV- related PTB)
    ≥10mm - Positive
  Controls: 0-9mm – Negative
    ≥10mm – Positive

Sensitivity: 10/47x100=21.28%

Specificity: 12/100x100=12.0%

Positive predictive value: 10/98x100=10.20%

Negative predictive value: 12/49/100x100=24.5%

Table 3 shows that among the cases, 91% had a positive TB serologic test compared with 26% in the control group, with a specificity of 74%. The positive- and negative-predictive values were 78% and 89% with efficiency of 82.5%, respectively.

Figure 3

Table 3. Values of measures of validity for the serologic test in study subjects

Serologic test	Subjects		Total
	Cases	Controls	
Positive	91	26	117
Negative	9	74	83
Total	100	100	200

Specificity: 91/100x100 = 91%

Sensitivity: 74/100x100 = 74%

Positive Predictive value: 91/117 x100= 77.8%

Negative Predictive Value: 74/83x100 = 89%

Efficiency: (91+74/91+26+9+74) x100 = 82.5%

Tables 4 and 5 show the relationship between serology and Mantoux test in both HIV/TB and TB only patients. The serological technique was positive in 28/29 (96.6%) patients who had positive Mantoux test and 21/24 (87.5%) patients with negative Mantoux.

Figure 4

Table 4. Relationship between serologic test and Mantoux reaction in patients with HIV/PTB

Serologic test for TB	Mantoux reaction (mm)		Total
	≥5	<5	
Positive	28	21	49
Negative	1	3	4
Total	29	24	53

- Legend: Positive = induration size ≥5mm (HIV/PTB)

  Negative = induration size <5mm (HIV/PTB)

Figure 5

Table 5. Relationship between serologic test and Mantoux reaction in patients with PTB only

Serologic test for TB	Mantoux reaction (mm)		Total
	≥10	<10	
Positive	35	7	42
Negative	3	2	5
Total	38	9	47

- Legend: Positive = induration size ≥10mm (PTB only)

  Negative = induration size <10mm (PTB only)

DISCUSSION

This study has shown that the prevalence of HIV among TB patients is high (53%). Those in the 25-29-year age group were found to be most affected by TB and more than 80% of the population studied was below the age of 50 years. This agrees with earlier reports from other developing countries.[17-22] The Mantoux reaction of 12% among the control group in this study could be attributed to previous exposure to mycobacteria.

Using the CDC criteria for the interpretation of TST,[23] 55% of the HIV/PTB group had a positive reaction compared with 79% in the PTB only group. The negative predictive value of about 71% of Mantoux reaction in HIV/PTB makes it an inadequate screening test in this group compared with 88% in those with PTB only. The high negative Mantoux reaction in the study population might be due to anergy from HIV infection or malnutrition as a little over 50% of the patients studied had HIV-co-infection. Previous researchers had also reported anergy in their populations.[24, 25]

The sensitivity (91%) and specificity (74%) of the serologic test accord well with previous studies with the native 38kDa antigen.[26] The high negative predictive value of this test (82.5%) makes it a potentially useful screening test to rule out active TB especially in HIV/PTB, where other diagnostic methods have failed. The sensitivity, specificity, and predictive values of the serologic test in this study were higher than those of Mantoux test in TB detection which is in keeping with the observation that in patients without significant sputum production and cutaneous anergy, the serologic test stands out as the "gold standard" superseded only by PCR in excluding tuberculosis.[27]

The resurgence of TB in the era of HIV pandemic makes early diagnosis for chemotherapy or prophylaxis imperative since 50% of those infected with HIV will develop TB.[28]

CONCLUSION

Both Mantoux and ELISA methods have been found to be useful in the diagnosis of TB, though the high level of anergy to the former, especially in the HIV-infected individuals has rendered this hitherto important test less effective. The serologic approach using ELISA in this regard is handy and therefore recommended for resolving this diagnostic dilemma.

References

1. Skvor J, Trnka Kugukovova Z. Immunoprofile studies in patients with pulmonary tuberculosis. Scand journ of Resp Dis 1979; 60: 148.
2. Davies PDO. The pathogenesis of tuberculosis. Post Grad

Doctor Africa 1984; 6(9):269.

3. Lester CF, Atwell RJ. The tuberculin skin reaction in active pulmonary tuberculosis. Am Rev Tuberc 1958; 78: 339-402.

4. Youmans GP Mechanism of immunity in tuberculosis. Pathobiol Annu 1979; 9:137-162.

5. Grange JM, Gibson J, Nassau E. Enzyme-Linked Immunosorbent Assay (ELISA): A study of antibodies to Mycobacterium tuberculosis in the IgG, IgA and IgM classes in tuberculosis, sarcoidosis and Crohn's disease. Tubercle 1980; 61:145-152.

6. Nassau E, Parsons ER, Johnson GD. The detection of antibodies to Mycobacterium tuberculosis by microplate enzyme-linked immunosorbent assay (ELISA) 1976; 57:67

7. Baldwin RW, Hand CN. Electrophoretic studies of the serum proteins in tuberculosis. Am Tuberc 1953; 68: 372-381.

8. Bovornkiti S. Serum protein changes in tuberculosis in humans, with reference to alfa 2 globulin. Am Rev Res Dis 1962; 85: 58-65.

9. Gilliand IC, Johnson RN, Stradling P, Abdel-Wahab EM. Serum proteins in pulmonary tuberculosis. Brit Med 1956; 1: 1460-1464.

10. Leggat PO. Serial serum protein changes in pulmonary tuberculosis. Brit J Tuberc Dis Chest 1957; 51: 139-145.

11. Seibert SB, Seibert MN, Atno PJ, Campbel HW. Variation in protein and polysaccharide content of sera in the chronic diseases, tuberculosis, sarcoidosis, and Carcinoma. J Clin Invest 1947; 26: 99-102.

12. Volk BW, Saifer A, Johnson LE, Oreskes I. Electrophoretic and chemical serum protein fractions in pulmonary tuberculosis. Am Rev Tuberc 1953; 67: 229-321.

13. Kardjito T, Grange JM. Immunological and clinical features of smear positive pulmonary tuberculosis in East Java. Tubercle 1980; 61: 231-238.

14. Charles Mantoux (www.whonamedit.com). Accessed on 12th December, 2007

15. Sokal JE. Measurement of delayed skin test responses. N Eng J Med 1975; 293: 501-502.

16. Galen RS, Gambino SR. Beyond normality-the predictive value and efficiency of medical diagnosis. New York. John Wiley and sons 1975:1.

17. Wokoma FS. Human Immunodeficiency Virus (HIV) status of adult Nigerian patients suffering from pulmonary tuberculosis. Nig Med Pract 1997; 34:22-24.

18. East Africa /British Medical Research Council Study. Controlled comparison of p aminosalicylic acid and thiacetazone in antituberculosis regimens. 3rd investigations report. Am Rev Resp Dis 1996; 64-76.

19. Diagnostic advances in tuberculosis- Clinical forum .Afr Health 1989; 11(3): 19.

20. Ekweani CN. Streptomycin and thiazinah in the treatment of pulmonary tuberculosis in Kaduna. A preliminary report. FMCP Dissertation 1989.

21. Ali-Gombe A. Factors affecting sputum conversion in patients with active pulmonary tuberculosis using the modified short therapy and the conventional short course therapy. FWACP Dissertation 1991.

22. Idigbe EO, Sofola TO, John E, et al. Trend of pulmonary tuberculosis in Lagos, Nigeria 1982-1992. Biomedical letters 1995; 51: 99-109.

23. Jones BA, Young SMM, Astoniskis D et al. Relationship of the manifestations of tuberculosis to CD4 cell counts in patients with HIV infection. Am Rev Resp Dis 1993; 148:1292.

24. Nash DR, Douglas JE. Anergy in active tuberculosis. Chest 1980; 77: 32.

25. Komstock GW, Livesay VT, Woolpert SF. The prognosis of positive tuberculin reaction in childhood and adolescence. Am J Epid 1994; 99.

26. Daniel TM. Rapid diagnosis of tuberculosis: laboratory technique applicable in developing countries. Rev infect Dis 1989: 11(2) 471-478.

27. Al Zahrani K, Al Jahdali H, Poirier L, Rene P, Gennaro ML and Menzies D. Accuracy and Utility of Commercially Available Amplification and Serologic Tests for the Diagnosis of Minimal Pulmonary Tuberculosis. Am J Respir Crit Care Med. 2000; 162 (4): 1323-1329.

28. DeCock KM. Tuberculosis control in Resource-poor setting with High rates of HIV infection. Am J Public Health 1996; 86: 1071 – 73.

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