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Mantoux reaction in patients with HIV-related pulmonary tuberculosis in Maiduguri, Nigeria

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Summary

Tuberculin skin testing is used for the identification of individuals with infection by *Mycobacterium tuberculosis* and other non-tuberculous mycobacteria. However, its value in immunosuppressed individuals due to human immunodeficiency virus (HIV) infection is controversial. This study was aimed at determining the relationship between Mantoux reaction and CD4+ cell counts; and whether the test can be used to predict CD4+ counts in patients dually infected with Human Immunodeficiency Virus and *M. tuberculosis*. Eighty patients, comprising 42 males (52.5%) and 38 females (47.5%) confirmed to be having antibodies to HIV who also had sputum smear positive pulmonary tuberculosis were recruited over a period of 16 months. They were Mantoux-tested with 0.1ml of 5TU of PPD which was interpreted thus: <5mm = negative, =5mm = positive. CD4+ counts were determined using Dynabeads technique. The ages of all the patients ranged between 18 and 55 years (mean \pm SD: 33.9 \pm 8.42 years). The males had a mean age of 35.4 \pm 7.7 years while that of the females was 29.6 \pm 5.3 years (P <0.05). The CD4+ counts ranged between 73 and 512 cells/ μ l with a mean of 235.05 \pm 112.8 cells/ μ l. Fifty seven (71%) patients had negative PPD tests while 23 (29%) tested positive. Of the 37 with CD4+ counts <200 cells/ μ l, 32 (86.48%) had negative reaction (<5mm) and 5 (13.51%) were positive (=5mm) as compared to those with CD4 counts =200 cells/ μ l, among whom 25 (58.13%) were negative and 18 (41.86%) were positive (P <0.05). The positive predictive value was low at 56.14%. The difference in mean indurations between those with CD4+ count <200 cells/ μ l versus those with CD4+ count =200 cells/ μ l was statistically significant (P <0.05). On the whole, Mantoux indurations were noted to weakly correlate positively with CD4+ counts (Pearson's correlation, r =+0.36, P =0.001). It was concluded that there is a weak positive correlation between Mantoux reaction and CD4+ cell counts and that the Mantoux test is a poor predictor of CD4+ cell count.

Keywords: HIV-related tuberculosis, Mantoux reaction, CD4+ counts, Nigerians

Résumé

Le test de mantoux est utilisé pour identifier les infections tuberculeuses et d'autres non-mycobactérienne. Cependant, son utilité sur les individus immunosuppressés due au virus du VIH/SIDA reste controversé. Cette étude avait

pour but déterminer la relation entre le test de Mantoux et le taux des cellules CD4+, et l'utilité de ce test pour prédire les patients ayant les individus séropositif ayant le *M. tuberculose*. Quarante-deux patients (42 (52.5% mâles et 38 (47.5%)) confirmés séropositifs et tuberculeux, étaient recrutés et suivit pour une période de 16 mois. Le test de Mantoux de 0.1ml de 5 TU était interprété comme <5mm = négative et >5mm positive le taux de cellules CD4+ était déterminé par la technique de Dynabeads. L'âge variait entre 18-55 ans avec une moyenne d'âge de 33.9 \pm 8.42 ans (P <0.05) le taux de cellules CD4+ variait entre 73-212 cellules/ μ l avec une moyenne de 235 \pm 112.8 cellules/ μ l. Vingt-trois (29%) était positive au test de mantoux, bien que 37 avaient le taux de cellules de CD4+ <200 cellules/ μ l, 32 (86.48%) étaient négative et 5 (13.5%) étaient positive comparant à ceux ayant un atax de CD4+ >200 cellules / μ l; parmi ceux-ci 25 (58.13%) étaient négative contre 18 (41.86%) positive (P <0.05). La valeur de predication positive était de faible de 56.14%. la différence en temps moyenne des taux de cellules CD4+ entre les 2 groupes était statistiquement significative (P <0.05). Il y avait une faible mais positive corrélation entre le test de mantoux et le taux positive des cellules CD4+ (r =0.36, P <0.001). Ainsi, le test de mantoux est une faible prédicteur du taux des cellules CD4+ chez les individus seropositif tuberculeux

Introduction

Tuberculin skin testing is a basic clinical procedure, originally used for identification of individuals with infection by *Mycobacterium tuberculosis* and non-specific mycobacterial infections [1-3]. Although skin testing with purified protein derivative (PPD) by the Mantoux method is a standard method of screening for tuberculosis (TB) infection, this method may be hampered by non reactivity to skin tests of persons who become immunosuppressed because of human immunodeficiency virus (HIV) infection.

In the past, a tuberculin reaction of 10mm was considered to be positive and indicated that the patient was infected. Because of increasing incidence of TB, especially among immunocompromised patients, the CDC, in 1990, issued new guidelines for interpretation of tuberculin reaction [4]. One-third to one-half of patients with acquired immune deficiency syndrome (AIDS) and TB may have an >10mm reaction to PPD but in patients with HIV infection and suspected TB, a 5mm reaction to PPD should be considered indicative of TB infection [5].

This paper reports the findings of a study correlating immune status (as determined by CD4+ counts) with Mantoux test and determining whether the Mantoux test can be used to predict CD4+ counts in patients with HIV-related pulmonary tuberculosis.

Methods

Study area

The study was carried out in the Department of Medicine, University of Maiduguri Teaching Hospital, Nigeria's Centre of Excellence for Immunology and Infectious Diseases. Maiduguri is the capital of Borno State in northeastern Nigeria with a population of about 0.8 million. It lies between latitudes 11.5° and 13.5°E in the Sudan Savannah. The hospital serves as a major referral center for the north-east sub region of Nigeria and the neighbouring countries of Cameroun, Niger and Chad Republics.

Study population

Eighty patients comprising 42 males and 38 females with newly confirmed HIV infection and sputum-smear positive pulmonary tuberculosis, seen consecutively at the medical outpatient department and medical wards of the University of Maiduguri Teaching Hospital, were studied over a period of 16 months from August, 2001 to November, 2002 after giving an informed verbal consent. Patients with chronic renal failure, chronic liver disease, and malignancies were excluded. Others excluded were patients on anti tuberculosis therapy for more than two weeks, antiretroviral drugs for more than one week, cytotoxics or steroids and patients with other debilitating diseases.

Patients were clinically evaluated by the authors at enrollment and parameters such as age, sex, weight, height, drug history and clinical history were recorded on a data sheet. Laboratory scientists in the Immunology Department of the center of excellence laboratories who were conversant with Mantoux testing administered 0.1ml of 5TU of phenol-preserved Tween-80 stabilized purified protein derivative (PPD) (Tubersol, Connaught Laboratories) intradermally over the volar aspect of the left forearm while a designated member of the group who was blinded to the result of HIV test read the transverse indurations at 72 hours using the ball-point technique of Sokol [6]. The CD4+ cell count and injection of PPD were carried out on the same day. The PPD was kept in the refrigerator when not in use. An induration =5mm was considered positive while that <5mm, negative [5].

Blood specimen for CD4+ count was collected during morning hours (10am-12noon) from a free flowing venepuncture and added to containers with Tri-potassium EDTA (anticoagulant) with the ratio 1.0-1.5mg of K₃EDTA to one ml of whole blood collected. Samples were adequately mixed to avoid clots. Blood samples were kept at ambient temperature avoiding refrigeration, and analysis carried out within 5 hours of specimen collection. Dynabeads CD4 (product N0. 111.05), a Dynal Biotech Product was used for the CD4+ count.

Results

The age of the patients ranged between 18 and 55 years (mean ± SD: 33.9 ± 8.42 years). The females were significantly younger (mean age: 29.63 ± 5.31 years) than the males (mean age of 35.42 ± 7.71 years) ($P < 0.05$). Table 1 shows the age and sex distribution of the patients. Most males were in the range of 30-39 years whereas females were mostly in the range of 25-34 years.

Table 1: Age and sex distribution of patients

Age (yrs)	Sex		Total (%)
	Male (%) n=42	Female (%) n=38	
15-19	1 (2.38)	3 (7.89)	4 (5.00)
20-24	5 (11.90)	7 (18.42)	12 (15.00)
25-29	5 (11.90)	13 (34.21)	18 (22.50)
30-34	10 (23.81)	8 (21.05)	18 (22.50)
35-39	12 (28.57)	2 (5.26)	14 (17.50)
40-44	5 (11.90)	3 (7.89)	8 (10.00)
45-49	2 (4.76)	1 (2.63)	3 (3.75)
50-54	1 (2.38)	0 (0.00)	1 (1.25)
55-59	1 (2.38)	1 (2.63)	2 (2.50)
Total	42 (100)	38 (100)	80 (100)

The CD4+ cell counts ranged between 73 and 512 cells/ μ l with a mean of 235.05 ± 112.83 cells/ μ l. Fifty seven (71.25%) patients had negative PPD tests while 23 (28.75%) tested positive. Of the 37 with CD4+ counts <200 cells/ μ l, 32 (86.48%) had negative reaction and 5 (13.51%) were positive as compared to the 43 with CD4+ cell counts =200 cells/ μ l, among whom 25 (58.13%) were negative and 18 (41.16%) positive ($P < 0.05$). The sensitivity, specificity and positive predictive values were 86.48%, 41.86%, and 56.14%, respectively. The difference in mean indurations between those with CD4+ count <200 cells/ μ l versus those with CD4+ count =200 cells/ μ l was statistically significant (3.26mm versus 1.51mm, $P < 0.05$) (Table 2). None of the patients with CD4+ counts <100 cells/ μ l had a positive Mantoux reaction. Table 2 also shows the relationship between tuberculin indurations and CD4+ T-Lymphocyte counts.

Tuberculin reactions were noted to weakly correlate positively with CD4+ count (Pearson's correlation, $r = +0.36$, $P = 0.001$).

Table 2: Diagnostic and predictive usefulness of Mantoux in HIV/TB patients

Mantoux Test to predict CD4+ counts	CD4+ cell count/ μ l as Gold standard of immunological depression		Total
	+ve test result; cell immune depression <200	+ve test result; immunologically competent <200	
Immune depression Positive (<5mm) test result	32	25	57
Immune depression Negative (<5mm) test result	5	18	23
Total	37	43	80

Sensitivity: 32/37 = 86.48%

Specificity: 18/43 = 41.86%

Positive predictive value: 32/57 = 56.14%

Discussion

Mantoux test is the preferred screening test for tuberculosis in most settings [7]. It was chosen in this study in-

stead of heaf test because of this reason and also because in previous studies in Nigeria [8,9], heaf test was negative and grade I positive in more than 50% of patients with sputum smear positive pulmonary tuberculosis. Furthermore, Mestit and Pollard [10] did not encounter any case of confirmed TB with negative Mantoux tuberculin test. Thus Mantoux test is more appropriate for screening for TB among Nigerians. However, purified protein derivative RT 23/Tween 80, currently recommended by the WHO and International Union Against Tuberculosis and Lung Disease (IUATLD) was not available for use in this study.

The age of the patients in this study agrees with the finding that HIV affects individuals in their active years [11]. Wokoma reported a similar age distribution among HIV-related PTB patients in Port Harcourt, southern Nigeria [12].

Most of those who tested positive for Mantoux (78.3%) had CD4+ count =200 cells/ μ l as compared to those who were negative ($p < 0.05$). This finding is in keeping with those of earlier reports [13,14]. The difference in mean indurations between those with CD4+ count <200 cells/ μ l versus those with CD4+ count =200 cells/ μ l was statistically significant ($p < 0.05$). None of the patients with CD4+ counts < 100 cells/ μ l reacted to PPD in this study which is in agreement with the findings of Jones *et al* [13]. This is not surprising, as pronounced reactions to tuberculin require a more vigorous CMI response as depicted by high CD4+ cell counts.

The high rate of negative Mantoux tests in this study is in keeping with those of other studies in Nigeria [15,16]. This may be due to the fact that most of our patients presented late with low CD4+ counts and hence poor CMI responses.

The rate of Mantoux negativity was unacceptably high among our patients with CD4 counts =200 cells/ μ l. Although a positive tuberculin test is widely accepted as an indication of past or present mycobacterial infection, true determinants are unknown for studies of the sensitivity and specificity of the tuberculin test among the general population, and the predictive accuracy of the test cannot be estimated. This may explain the low predictive value of the test in our patients (56.14%).

Dosunmu [17] in southwestern Nigeria reported that pulmonary tuberculosis cases and controls tended to have a large tuberculous reaction sizes with the tuberculous cases having a greater tendency, suggesting that this test lacks specificity. Although his study subjects were not HIV-infected, this study also shows that the test equally lacks specificity in patients with HIV-associated TB with a specificity of 41.9%.

Conclusion

A weak positive correlation exists between Mantoux test and CD4+ counts. The Mantoux test was also found to be a poor predictor of CD4 counts. However, larger studies that will include controls are required to further discern the usefulness of this test in the diagnosis of TB and predicting CD4+ cell counts. This was not possible in this study because of the high cost of CD4+ kits.

Based on the results of this study the Mantoux test cannot be recommended as a routine diagnostic, and

predictive, tool for tuberculosis and CD4 + count, in patients with HIV-associated TB.

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