



Original Research Article

Mantoux Test in HIV Infected Patients in Association with CD₄ Cell Count

S.Madhavi*, S.Shazia Parveen and M.Shravani

Department of Microbiology, Bhaskar Medical College and Hospital, Yenkapally, Moinabad, R.R.District, Telangana state-500075, India

*Corresponding author

A B S T R A C T

Keywords

TST,
Mantoux
test,
HIV,
CD4
cell count

The tuberculin skin test (TST) done with purified protein derivative (PPD) by the Mantoux method, is useful for screening for TB infection in vivo. The objective of this study is to determine the frequency of Mantoux test positive in those infected with human immunodeficiency virus (HIV) and to investigate its association with CD4-T cell count, to know the percentage of people with HIV suffering with tuberculosis and at risk for tuberculosis. The study group consisted of 40 diagnosed HIV positive patients both newly diagnosed and old cases. Mantoux test was performed and the CD4 lymphocyte counts estimated by flow cytometry in 40 HIV-positive patients. Out of the 40 HIV patients 14 were Mantoux positive and 26 were Mantoux negative. Old HIV positive patients were mostly Mantoux positive though the CD4 cell counts in both groups were almost similar. Mantoux test positivity decreased with the decreasing CD4 cell counts. And it was found that the subjects with CD4 cell counts between 0-200 were mostly Mantoux negative. Mantoux test can be used as a diagnostic tool for decreasing immunity in HIV patients but to predict Tuberculosis in HIV patients more tests are required.

Introduction

Human Immunodeficiency Virus (HIV) is a lot like other viruses, but there is an important difference over time, our immune system can clear most viruses out of our body but not HIV. HIV can hide for long periods of time in the key part of immune system – T cells or CD4 cells. HIV invades them, uses them to make more copies of itself, and then destroys them. Early in the course of the disease, the body can make more CD4 cells to replace the ones that have been damaged by HIV.

Eventually, the body can't keep up and the number of functioning T-cells decreases. As more and more CD4 cells become damaged, the immune system becomes more and more weakened. Eventually, the weakened immune system leaves the body at risk for illness and infections. Acquired Immunodeficiency Syndrome is the final stage of HIV infection. People at this stage of HIV disease have badly damaged immune systems, which put them at risk for opportunistic infections (OIs). Tuberculosis

(TB) is one of the commonest opportunistic infection in patients with HIV/AIDS. Sowmy et al (2008) Further, there has been an increase in rates of drug resistant tuberculosis, including multi-drug (MDRTB) and extensively drug resistant TB(XDRTB), which are difficult to treat and contribute to increased mortality. The number of new TB cases has tripled in high HIV prevalence countries in the last two decades but has shown a slight decline in 2009. TB is the leading cause of death among people living with HIV. It is also the most common presenting illness among people living with HIV, including those who are taking antiretroviral treatment. Tuberculin skin testing is a reliable tool for the detection of tuberculosis infection and its eventual prevention. False-negative tests may occur in individuals with a compromised immune system, including those with human immunodeficiency virus (HIV) infection, persons taking immunosuppressive drugs (e.g. corticosteroids), severely malnourished and the elderly. Nevertheless it is still useful in detecting infection in those who are in close contact of patients with tuberculous disease. Once a skin test is found to be reactive, further diagnostic studies should be done to rule out tuberculosis disease. Once ruled out, preventive therapy with isoniazid or a combination of drugs should be instituted. TB cannot be eliminated until the importance of preventive therapy is recognized by all [Hanson CA et al.(1998)].

The tuberculin skin test (TST) done with purified protein derivative (PPD) by the Mantoux method, which is highly sensitive but less specific than other cell-based methods, is useful for screening for TB infection in vivo. Tuberculin skin reactivity is dependant on cytokine mediated cellular immunity. It is reported to be less sensitive among immune compromised individuals.

Its prevalence of reactivity is reported to be positively correlated with absolute counts of CD4+ and total lymphocytes. TB is endemic in our country and vaccination with Bacillus Calmette –Gurine (BCG) is routinely given to all newborns in India. Therefore, age is a factor which could interfere with the size of induration in PPD test. PPD-positive HIV infected patients are recommended to have isoniazid preventive therapy (IPT) against TB. In a recent study of HIV-infected patients by Frank G. Cobelens et al (2006) the prevalence of tuberculin reactivity varied directly and that reduction in sensitivity of the TST is predominantly caused by anergy that occurs as an all-or-nothing phenomenon. At present, limited information is available on delayed type hypersensitivity (DTH) skin test responses in HIV-infected patients. Considering the above points and regarding the importance of this issue, this study was conducted to investigate the existing problem, the frequency of PPD positivity and its correlation with CD4+ T cell count. In HIV and TB co-infected patients, a reduction in PPD reactivity has been reported as CD4 count falls as it happens in patients with advanced HIV infection. The objective of this study is to determine the frequency of Mantoux test (Tuberculin skin test(TST) positive in those infected with human immunodeficiency virus (HIV) and to investigate its association with CD4-T cell count, To know the percentage of people with HIV suffering with tuberculosis and at risk for tuberculosis.

Materials and Methods

The study was carried out on subjects attending the ICTC of Bhaskar General Hospital, Yenkapally, Moinabad (m), R.R. Dist in co-ordination with the Department of Microbiology. The study group consists of 40 diagnosed HIV positive patients, The

study included – Performing TST in 40 diagnosed HIV-positive patients, newly diagnosed and old cases also and to measure the CD4 lymphocyte counts by flow cytometry. HIV positivity was diagnosed and confirmed by doing 3 tests according to NACO strategy IV in the patients attending the ICTC of the hospital.

The three tests 1) Combaids test
2) Pareekshak HIV ½ Triline card test
3) Pareekshak HIV TRI Spot test.

The principle of the Combaids test is dot immunoassay intended for the qualitative detection of IgM/ IgG antibodies to the HIV type 1 and type 2 in whole blood, serum or plasma THE PAREEKSHAK HIV ½ triline card test is an immuno chromatographic baser assay for the detection of antibodies to HIV -1, HIV-2 in human serum or plasma. This uses antigens like gp 41, gp-120, gp-36. The Pareekshak HIV tri spot test is an immuno concentration based assay for the detection of antibodies to HIV-1, HIV-2 in human serum or plasma. This uses antigens like gp-120, gp-47.

Mantoux Test and CD4 cell counts were estimated in all the patients tested positive by all the three tests .

TST was performed by the following method –

Mantoux Test

[DTBE - Mantoux TB Skin Test Faciliator Guide - Part 1: Administering]

Test Administration

0.1 ml of 5 Tuberculin Units PPD was given intradermally. The results were read between 48 and 72 hours after giving the injection. If more than 72 hours has elapsed

and there is not an easily palpable positive reaction, the test was repeated on the other arm and read at 48 to 72 hours after the second administration.

The induration was measured - not erythema. It was measured and reported as millimeters of induration. Generally: 5 or more millimeters induration was considered positive [Frank G. Cobelens et al.(2006)] for the highest risk groups, such as: Persons with HIV infection Persons who have had close contact with an infectious tuberculosis case Persons who have chest radiographs consistent with old, healed tuberculosis Negative Reactions Reactions below the cutting point are considered negative.

CD4 Cell Count by Flow Cytometry

Blood samples were collected from patients. These blood samples were collected in EDTA and analyzed with in 24h using `lyase-no-wash` procedure [Greve, Bet al.2003] Absolute lymphocyte counting were performed by a FACS Calibur Bector Dickinson immuno cytometry system) operating with Tru COUNT Tubes (30 preloaded with a number of beads(46,295 beads per tube) cell concentration and calculated with formula (number of events in the region containing cell population |number of events in region containing beads)x(46,295|test volume[50microl])

Reagents

During the bead-based control procedure on the FACS Calibur, TRU COUNT Tubes were used in combination with MULTI TEST reagents to obtain absolute CD4 counts On FACS Calibur ,the gating strategy recommended by manufacturer was used, with the threshold set for red fluourescence (CD45)in a single tube.

Normal CD4count-500-1600 A PERSON with CD4 count-200 or a CD4%-less than 14% is considered to have AIDS.

Results and Discussion

Mantoux test was done in 40 diagnosed HIV-positive patients, including newly diagnosed and old(previously diagnosed) cases and CD4 lymphocyte counts were estimated by flow cytometry. Out of the 40 patients 16 (40%) were females and 24 (60%) were males .Average age of the patients was 29.65 years (range3-55). Age of the patients and TST results are shown in Table 1. Out of total 40 subjects 14 (35%) were Mantoux positive and 26 (65%) were Mantoux test negative. Out of 14 subjects who were Mantoux test positive 6 (42%) were females and 8 (58%) were males.

In our study of 40 patients , 10 were old patients who were Mantoux positive which is of 25%,, 8 were old who are Mantoux negative , which was 20%, 4 newly diagnosed cases were Mantoux positive which was 10%, 18 are new who are Mantoux negative which was 45%. As shown in Table 2.This shows that most of the old HIV subjects are seen to be Mantoux positive.

Out of 40 subjects 14 were old subjects with CD4cell counts 0-500 and 4 subjects were old subjects with CD4 cell count 501-1100. and 16 were new subjects with CD4 CELL COUNTS 0-500 AND 6 were new with CD4cell counts 501-1100.This is shown in Table 3.

Table4 shows that 6 patients with CD4 range of 0-500 were Mantoux positive out of 30 patients. And 8 patients with CD4 range 501-1100 were Mantoux positive out of 10.This shows that the subjects with more CD4 counts are mostly Mantoux positive

and the subjects with less CD4 counts are mostly Mantoux negative.

Table 5 shows that out of 10 subjects who are on ART 6 were Mantoux positive and out of 30 subjects who are not on ART 8 were Mantoux negative.

This shows that the subjects who were on ART are mostly Mantoux positive that is out of 10 subjects 6 were positive and out of 30 subjects who were not on ART only 8 were negative .

According to table 6, 14 subjects with CD4 cell counts between 0-200 are having a Mantoux range of 0mm (includes Mantoux negative subjects) . 10 subjects with CD4 count 201-500 were having a Mantoux range of 0mm. 2 subjects with CD4 count 501-1000 were having a Mantoux range of 0mm. 4 subjects with CD4 count between 0-200 are having Mantoux range 1-5mm. And 2 subjects with CD4 count 201-500 were having Mantoux range 1-5mm.6 subjects with CD4 count 501-1000 were having Mantoux range 6-10mm. and 2 subjects with CD4 count 1001-1500 were having Mantoux range 11-20mm. This shows that with increasing CD4 counts Mantoux reading is increasing.

The present study group consisted of 40 patients who were old and new HIV positives. In them 14 were Mantoux positive and 26 were Mantoux negative.

1) The old subjects with HIV who were detected to have HIV infection years ago were seen to be mostly TST positive than the subjects who were recently detected. This may be due to having HIV for a long period of time which decreases the immunity to a greater extent and prone for reactivation of latent tuberculosis or may be easily susceptible to the new tuberculosis

infection or may be due to ART(anti retro viral therapy).The subjects who were recently HIV detected may have less duration with decreased immunity so that they are not easily susceptible to the tuberculosis infection and also they are not on ART. This can be shown by less number of Mantoux positives in recently detected HIV subjects. This slightly deviates with the study of Col MPS Sawhney, Maj Gen YK Sharma (2006) who stated that in countries endemic for tuberculosis, the infection is likely to occur early, when the body immunity is still intact (new), giving rise to a strongly positive tuberculin reaction. In advanced HIV disease (old), when there is increased immune suppression, the body may not be able to produce adequate TST results. In our study though the TST Results were not adequate i.e., not more than 10mm in old subjects but were mostly positive in them .Thus this does not show any thing about immune competence but shows only about the duration of decreased immune competence .But when we consider immune competence our study coordinates with their study.

2) In our study both old and new subjects had CD4 cell counts between 0-500 and 501-1100 though old and new subjects had CD4 ranges between 0-500 old subjects are mostly Mantoux positive.

3) The subjects with CD4 cell counts more than 500 are mostly Mantoux positive's than the subjects with CD4 cell counts less than 500. This is because Mantoux test is a type of hypersensitivity reaction which depends on CD4 cell count. And as the CD4 cell count decreases the Mantoux reactivity also decreases. This coincides with the study of Frank G. Cobelens et al (2006) who also showed that with decreasing immune competence the Mantoux reactivity also decreases. Another study done by Yusuph

H al(2005). 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. 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.

4) The subjects who are on ART are mostly Mantoux positive than the subjects who are not on ART. This is because the subjects who are on ART will have increased CD4 cell counts due to the therapy. And increased CD4 cell counts will increase the reactivity to Mantoux test.

5) As the CD4 cell count is decreased the diameter of the induration in Mantoux test is also decreased. The subject with CD4 cell count more than 1000 had a diameter of 11-20 mm in Mantoux test, the subject with CD4 cell count 500-1000 had a reaction of 5-10mm and the subject with CD4 cell count 0-500 had a diameter of 0-5mm.This is due to as the CD4 count is decreased the Mantoux reactivity decreases which lead to decreased induration. This coincides with the study done by Dr. Bhavin Dalal et al (2002) who also showed that the range of Mantoux reactivity decreases with the decreasing CD4cell counts .

In conclusion, Mantoux positivity is seen more in old HIV cases than in newly diagnosed HIV cases. The difference in CD4 counts between old and newly diagnosed cases is not very significant. Subjects with more CD4 counts are mostly Mantoux positive and the subjects with less CD4 counts are mostly Mantoux negative, subjects with CD4 cell counts less than 100-200 are with nil induration. Subjects who are

on ART are mostly Mantoux positive. Mantoux test positivity decreases with the decreasing CD4 cell counts. With increasing CD4 counts Mantoux reading is increasing i.e. the diameter in mm. Mantoux test can be used as a diagnostic tool for decreasing immunity in HIV patients but to predict Tuberculosis in HIV patients more tests are required. HIV cases without clinical evidence of tuberculosis with TST positivity of 10mm or more and all cases of negative reaction to TST who have CD4 counts lower than 200/cmm are at risk of developing

tuberculosis and should be administered chemoprophylaxis. In HIV patients the Mantoux cut off should be considered up to 2mm as CD4 counts decrease and for patients with CD4 count <200 Mantoux should not be taken as a reliable test and other tests are to be considered like x-ray, sputum positivity. Hence it is recommended that all cases with TST of = or > 10 mm and cases with nil induration with CD4+ count of <200/cmm should be considered as high-risk for developing tuberculosis and should be administered chemoprophylaxis.

Table.1 Age and Tuberculin Test in HIV-Positive Patients

| AGE | 0MM | 1-2MM | 3-5MM | 6-9MM | 10-15MM | >15MM |
|-----------|-----|-------|-------|-------|---------|-------|
| 1-20 YRS | - | - | - | - | 4 | 2 |
| 21-30YRS | 16 | - | 2 | - | - | - |
| 31-40YRS | 6 | 2 | 2 | - | 2 | - |
| 41-50 YRS | 2 | - | - | - | - | - |
| 51-60 YRS | 2 | - | - | - | - | - |

Table.2 Showing Mantoux Positive and Negative in Old and New Patients

| | OLD % | NEW% | TOTAL |
|---|---------|---------|-------|
| MANTOUX POSITIVE | 10(25%) | 4(10%) | 14 |
| MANTOUX NEGATIVE | 8(20%) | 18(45%) | 26 |
| | 18 | 22 | 40 |
| P VALUE: 0.013685 Level of significance: significant | | | |

Table.3 Showing CD4 Cell Counts in Old and New Patients

| | OLD | NEW | TOTAL |
|--|-----|-----|-------|
| CD4 -0-500 | 14 | 16 | 30 |
| 500-1100 | 4 | 6 | 10 |
| | 18 | 22 | 40 |
| P VALUE:0.713628 Level of significance: Not Significant | | | |

Table.4 Showing Mantoux Positive and Negative in In Patients with CD4 Count Between 0-500 and 501-1100

| | 0-500 | 501-1100 | |
|---|----------|----------|----|
| MANTOUX POSITIVE | 6(15%) | 8(20%) | 14 |
| MANTOUX NEGATIVE | 24(60%) | 2(5%) | 26 |
| | 30 | 10 | 40 |
| P VALUE: 0.000571 Level of significance: Significant | | | |

Table.5 Showing Mantoux Positive and Negative In Patients on Art and not on Art

| | ART | NON ART | TOTAL |
|---|---------|----------|-------|
| MANTOUX POSITIVE | 6 (15%) | 8 (20%) | 14 |
| MANTOUX NEGATIVE | 4 (10%) | 22 (55%) | 26 |
| | 10 | 30 | 40 |
| P VALUE: 0.055633 Level of significance: Significant | | | |

Table.6 Showing Mantoux Test Range(In Mm) In Patients with CD4 Counts 0-200,201-500, 501-1000, 1001-1500

| | CD4-0-200 | 201-500 | 501-1000 | 1001-1500 | TOTAL |
|---|-----------|-----------|----------|-----------|-------|
| MANTOUX RANGE: 0mm | 14 (35%) | 10 (25%) | 2 (5%) | 0 | 26 |
| 1-5mm | 4 (10%) | 2 (5%) | 0 | 0 | 6 |
| 6-10mm | 0 | 0 | 6 (15%) | 0 | 6 |
| 11-20mm | 0 | 0 | 0 | 2 (5%) | 2 |
| | 18 | 12 | 8 | 2 | 40 |
| PVALUE:3.00848E11, Level of significance :Significant | | | | | |

Acknowledgement

This work was carried out by M.Shravani under the guidance of Dr.Madhavi.S as a part of the short term studentship project (STS) by the Indian Council of Medical Research during June 1st, 2012 to July 31st, 2012.in ICTC and Department of Microbiology, Bhaskar General Hospital, Yenkapally, Moinabad, R.R.dist , T.S, India.

References

- Bhavin Dalal, Kamal Goplani.2002. Correlation of Tuberculin Test & CD4 Count in HIV Positive Patients with Pulmonary & extra pulmonary Tuberculosis, RCJournal.com » Past OPEN FORUM Abstracts »
Col MPS Sawhney, Maj Gen YK Sharma, Significance of Tuberculin Testing in HIV Infection: AnIndian

- Perspective, MJAFI 2006; 62 : 104-107
- DTBE - Mantoux TB Skin Test Faciliator Guide - Part 1: Administering
- Frank G. Cobelens, Saidi M. Egwaga, Tuberculin Skin Testing in Patients with HIV Infection:Limited Benefit of Reduced Cutoff Values, *Clin Infect Dis. (2006) 43 (5): 634-639*
- Greve, B., Cassens, U., Westerberg, C., Ghde, W., Sibrowski, W.,Reichelt, D., 2003. A new no-lyse, no wash flow-cytometricmethod for the determination of CD4 T cells in blood samples. *Transfus. Med. Hemother.* 30, 8—13.
- Hanson CA, Reichman LB. Tuberculosis skin testing and preventive therapy. *Semin RespirInfect* 1989; 4: 82-8.
- Soumya Swaminathan and G. Narendran, HIV and tuberculosis in India, *Journal of Biosciences* ,Volume 33, Number 4 (2008), 527-537
- Yusuph H, Garbati MA, Mantoux reaction in patients with HIV-related pulmonary tuberculosis in Maiduguri, Nigeria. *Afr J Med Med Sci.* 2005 Jun;34(2):125-8.