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THE OCCURRENCE OF CLINICALLY ACTIVE TUBERCULOSIS AMONG HIV-INFECTED PERSONS IN MUMBAI, INDIA.

By

[RAJIV S. HIRA]

[Degree to be awarded: MPH]

[Department of Epidemiology]

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[PATRICK SULLIVAN, PhD]

Faculty Thesis Advisor

Abstract Cover Page

THE OCCURRENCE OF CLINICALLY ACTIVE TUBERCULOSIS AMONG HIV-INFECTED PERSONS IN MUMBAI, INDIA.

By

[RAJIV S. HIRA]

[M.B.B.S.]

[Maharashtra University of Health Sciences, Nashik, India]

[2011]

Faculty Thesis Advisor: [PATRICK SULLIVAN, PhD]

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Abstract

THE OCCURRENCE OF CLINICALLY ACTIVE TUBERCULOSIS AMONG HIV-INFECTED PERSONS IN MUMBAI, INDIA.

By [RAJIV S. HIRA]

Objective: To determine the CD4 count and HIV-1 viral load in HIV-infected persons in Mumbai, India developing incident *Mycobacterium tuberculosis* (MTB).

Patients &Methods: The database was from a prospective case-control study conducted at the teaching hospital of the MGM University, Mumbai during pre-ART era (2003-2004). It comprised of a purposive sample of 113 HIV-positive and 32 HIV-negative individuals with incident diagnosis of MTB; all adult, non-pregnant patients naïve to anti-tuberculosis treatment and ART. Investigations among other tests included Polymerase Chain Reaction test for MTB (TBPCR), Lowenstein Johnson (LJ) culture, CD4/CD8, and HIV-1 viral load count. Both bi-variate and multivariate analyses were performed using EpiInfo setting statistical significance at $p<0.05$ (2-tailed).

Results: The mean age of the cohort was 33.5$\pm$ 9.9 years. The ratio of male: female was 4:1. Among HIV-positive patients, the median CD4 counts were 340 cells/ml and viral load was log 0.6/ml. By comparison, for HIV-negative patients, the median CD4 counts were 758.5 cells/ml and viral load was log 0/ml. Between HIV+TBPCR+ (n=57) and HIV+TBPCR- (n=56) categories, the median CD4 count of 321 vs 480 cells/ml (p=0.03),

median CD8 count of 480 vs 686 cells/ml (p=0.05), and HIV-1 viral load of log 1.1 vs 0.30 (p<0.001) were higher in HIV+TBPCR+ category. However, between HIV-TBPCR+ and HIV-TBPCR- categories, variables including the median CD4 count of 750 vs 770 cells/ml (p=0.25) and the median CD8 count of 633 vs 690 cells/ml (p=0.28) were not statistically different.

The performance of both MTB confirmatory tests, TBPCR and LJ culture ('gold standard'), was not statistically different between all four groups. Regression analysis revealed a strong correlation between HIV infection status and TBPCR result even after adjusting for CD4 (F=44.97; p=0.02), adjusting for viral load (F=18.18; p=0.05), and when adjusting for both CD4 and viral load (F=31.02; p=0.009).

Conclusions: Incident MTB occurred in HIV-positive patients in Mumbai at median CD4 count of 321 cells/ml and viral load of log 1.1 particles/ml. With new WHO guidelines of 2013 recommending initiation of ART in HIV-positive patients at CD4 count <500 cells/ml, it may also help reduce occurrence of incident cases of MTB substantially.

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CHAPTER I:

THE OCCURRENCE OF CLINICALLY ACTIVE TUBERCULOSIS AMONG HIV-INFECTED PERSONS IN MUMBAI, INDIA.

Hypothesis:

Evidence from African studies suggests that HIV-positive persons developed clinically active MTB when their CD4 counts fell below 350 cells/ml (1). Based on this evidence, authors analyzed a data-set to test the hypothesis that HIV-infected persons in Mumbai, India also develop clinically active tuberculosis when their CD4 counts fall below 350 cells/ml. Consequently, if hypothesis is proved, the HIV-positive persons in Mumbai are likely to have dual benefits from the new WHO guidelines on anti-retroviral treatment, 2013 that suggests starting ART at CD4 cut-off count of 500 cells/ml; the primary benefit will be the effect of ART against HIV-1, and an added benefit will be averting activation of MTB because crucial cut-off level of CD4 <350 cells/ml will be avoided by ART initiation at a higher level of CD4 <500 cells/ml.

Primary Study Objective:

To determine the level of CD4 cells and HIV-1 viral count in HIV-infected persons with incident tuberculosis.

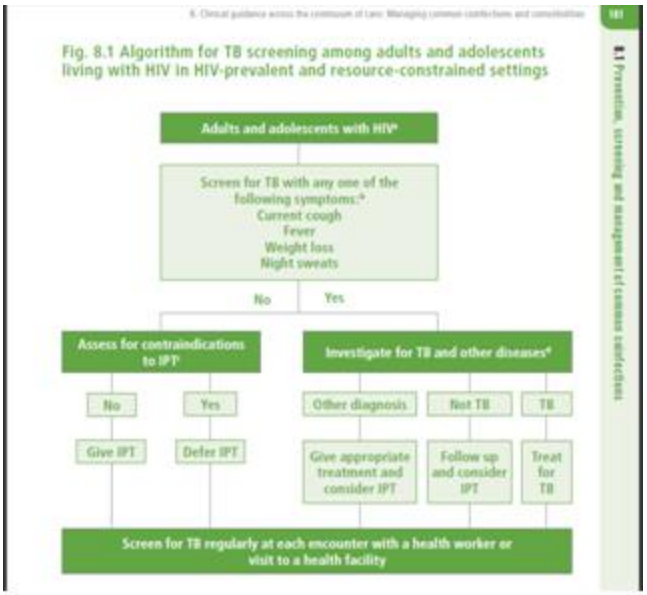
Background:

The World Health Organization (WHO) estimates that over 2 billion individuals globally are infected with Mycobacterium Tuberculosis (MTB) (1). While MTB continues to be a major cause of morbidity and mortality in developing countries, there has been resurgence even in developed countries where it was earlier successfully contained (2). The situation is further compounded by emergence of multi-drug resistant (MDR), extremely drug resistant (XDR), and total drug resistant (TDR) MTB (3,4). With the advent of the HIV/AIDS pandemic and its consequent immune suppression, the co-infection of TB/HIV is now recognized as a lethal combination (5). Such co-infection with MTB generally occurred in at least 60-70% of HIV-infected individuals during their life-time in pre-ART era, often leading to their rapid HIV-disease progression and death (1, 5).

WHO updated the guidelines for initiation of anti-retroviral treatment (ART) for developing countries in 2013 that suggest the CD4 count cut off count of <500 cells/ml based primarily on therapeutic benefits against HIV-1(6). However, these guidelines will also have an added impact on suppressing activation of tuberculosis in HIV-infected persons since there is evidence from studies in Africa that most tuberculosis in HIV-infected persons occurs when their CD4 cell count falls below 350 cells/ml. A dataset of a study conducted in Mumbai was analyzed to determine the level of CD4 cells and HIV-1 viral count in HIV-infected persons with incident tuberculosis.

Literature Search:

Among people living with HIV, TB is the most frequent life-threatening opportunistic infection and a leading cause of death (1). WHO Guidelines state "ART should be provided to all people with HIV with active TB disease irrespective of their CD4 counts (7). HIV care settings should implement the WHO *Three I's* strategy: intensified TB case-finding, isoniazid preventive therapy (IPT) and infection control at all clinical encounters (6). Adults and adolescents living with HIV should be screened for TB with a clinical algorithm; those who report any one of the symptoms of current cough, fever, weight loss or night sweats may have active TB and should be evaluated for TB and other diseases (Fig. 8.1 WHO guidelines)*(7). "Xpert MTB/RIF should be used as the initial diagnostic test in individuals suspected of having HIV-associated TB or multidrug-resistant TB .TB patients with known positive HIV status and TB patients living in HIV-prevalent settings should receive at least six months of rifampicin treatment regimen. Tuberculin skin test (TST) should be used to identify HIV-positive patients for INH prophylaxis treatment (IST)" (8).*



A study conducted between 1994 and 2001 before combination of anti-retroviral treatment was introduced in India showed that, of the 1820 patients with HIV infection, 410 (23%) presented with severe weight loss of > 10% of body weight within the preceding month. Of these 410 patients, 176 (43%) had tuberculosis, 94 (23%) had chronic diarrhea, and 89 (22%) had recurrent fever. Among 176 patients with tuberculosis, the following types of HIV-associated tuberculosis were seen: 115/176 (66%) had pulmonary tuberculosis and 49/176 (28%) had extra-pulmonary tuberculosis; of these 49 cases with extra-pulmonary tuberculosis 33 (18%) had disseminated tuberculosis and 12/176 (7%) had both pulmonary and extra-pulmonary involvement. In the group as a whole, 45/176 (25%) cases had disseminated tuberculosis. Clinical features of HIV-associated tuberculosis in decreasing order of frequency were chronic fever, chronic cough, lymphadenopathy and hepatosplenomegaly. The Mantoux skin test was significantly anergic among patients with extra-pulmonary and disseminated tuberculosis

($p = 0.001$). Thus, there was a significant correlation between severe weight loss and tuberculosis (RR 17.5), chronic diarrhea (RR 12.8) and recurrent fever (RR 4.5). The diagnostic value of the Mantoux skin test among HIV-associated tuberculosis is reduced, more so among those with extra-pulmonary and disseminated forms (9).

In a study conducted in Spain, an interaction was demonstrated between the CD4 count and the tuberculin skin test (TST) for the development of tuberculosis (TB) in HIV-infected patients (10). The study included 1824 patients starting on HAART, 339 (18.6%) of whom were TST-positive. After a median 473 days, 45 cases of TB had occurred (1.9 cases per 100 person-years, 95%CI 1.38-2.54). Among TST-positive patients, the rate of developing MTB was not affected by their CD4 count, irrespective of whether CD4 count was above or below 200 cells/ml (HR 1.37, 95% CI 0.44-4.21). The risk of developing TB increased significantly among female patients with a positive TST (HR 2.81, 95%CI 1.11-7.15). By contrast, in the TST-negative group, the risk was significantly higher in patients with CD4 count < 200 cells/μ l (HR 16.64, 95%CI 2.16-127.6). Thus, TST-positive patients are at high risk of developing TB, irrespective of CD4 count. However, there is a caveat for TST-negative patients due to anergy that is associated with advanced immune suppression i.e. that the TST-negative can be a false negative result, and a marker of severe immune suppression. Thus, irrespective of whether their CD4 count is < 200 cells/μl, TST-negative can be at an appreciable risk of developing the disease (10).

The absolute number of circulating CD4 cells in HIV infected patients have in large patient series been acknowledged as the strongest, single predictive factor of clinical deterioration [11-13]. In individuals with latent *Mycobacterium tuberculosis* infection,

CD4 depletion accelerates the progression from latent infection to active tuberculosis (TB), which, in turn, is believed to further fuel HIV replication rates due to elevated levels of pro-inflammatory cytokines [14]. TB by itself has also been associated with transitory lymphopenia including the CD4 positive cell lines [15,16]. A recent, retrospective study from Italy showed an impaired immune recovery in TB/AIDS cases compared to AIDS caused by other co morbidities which seemed not the be retrieved even after 3 years and despite access to efficient antiretroviral therapy (ART) [17]. A prospective cohort of newly diagnosed pulmonary TB patients from Tanzania reported a large dataset of 16-- studying the CD4 lymphocyte dynamics during TB treatment; both in HIV- uninfected and HIV-infected TB patients who all were ART-naïve (18). Their mean CD4 count of 285 cells/μl (95% CI 269,301) of the HIV-infected TB patients is in conformity that these were patients with moderately progressed HIV infection and recruited from outpatient clinic settings. There was the decrease in circulating CD4 lymphocytes induced by TB even before the diagnosis was made. The pattern was the same for both TB patients with and without HIV co-infection. HIV-uninfected PTB patients had significantly lower CD4 levels than healthy controls at baseline and did not reach the same levels of circulating CD4 cells even after 5 months of TB treatment. This could either be explained by continued sequestering of cells to the lungs or due to apoptosis and persistent regulatory stimuli even at this late stage towards the end of treatment [19-21]. The authors also found an impaired immune recovery of these patients compared to non-TB HIV patients which was persistent even after 3 years [17] and an association to delay in viral suppression in the HIV-TB patients group. However, the HIV patients without TB when put on ART within the first 2 months or from 2-5 months,

showed an increase in CD4 counts of 69 (95% CI: 22; 117) and 110 (95% CI: 52; 168), respectively (18).The HIV-infected TB patients who were put on ART at the time of TB diagnosis likewise did not increase their pool of circulating CD4 cells during the 5 months observation and treatment period (18, 22).

The results of three randomized, controlled trials (SAPiT, STRIDE, and CAMELIA) demonstrate that, for HIV/TB co-infected patients with advanced immunosuppression, the survival benefit of starting ART within the first 2 weeks of TB therapy outweighs the risk for immune reconstitution inflammatory syndrome and other adverse events (23). One logistical challenge of implementing these findings is that patients with suspected HIV/TB co-infection will need to have their CD4-cell counts measured at the time of sputum microscopy. Such a major barrier could unnecessarily delay the global application of research findings that would undoubtedly save lives (24).

A study conducted in Mumbai, India to assess the diagnostic value of PCR targeted to Insertion Sequence (IS) 1081 in peripheral blood of HIV-infected individuals established that PCR targeted to IS 1081 was a valuable test for early diagnosis of TB from peripheral blood at an early point of TB activation when most patients (>85%) did not produce other traditional specimens such as the sputum and/or pleural fluid. A cohort of 129 individuals was recruited for this purpose. TB PCR assay was compared with the ‘gold’ standard, namely the LJ culture. Overall, the sensitivity of PCR was 83.3% and specificity was 97.1%. Following treatment with rifampicin-contain anti-TB treatment, TB PCR+ patients converted to TB PCR negative between 6-8 weeks (25).

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CHAPTER II: Manuscript

Abstract

THE OCCURRENCE OF CLINICALLY ACTIVE TUBERCULOSIS AMONG HIV-INFECTED PERSONS IN MUMBAI, INDIA.

By [RAJIV S. HIRA]

Objective: To determine the CD4 count and HIV-1 viral load in HIV-infected persons in Mumbai, India developing incident *Mycobacterium tuberculosis* (MTB).

Patients &Methods: The database was from a prospective case-control study conducted at the teaching hospital of the MGM University, Mumbai during pre-ART era (2003-2004). It comprised of a purposive sample of 113 HIV-positive and 32 HIV-negative individuals with incident diagnosis of MTB; all adult, non-pregnant patients naïve to anti-tuberculosis treatment and ART. Investigations among other tests included Polymerase Chain Reaction test for MTB (TBPCR), Lowenstein Johnson (LJ) culture, CD4/CD8, and HIV-1 viral load count. Both bi-variate and multivariate analyses were performed using EpiInfo setting statistical significance at $p<0.05$ (2-tailed).

Results: The mean age of the cohort was 33.5$\pm$ 9.9 years. The ratio of male: female was 4:1. Among HIV-positive patients, the median CD4 counts were 340 cells/ml and viral load was log 0.6/ml. By comparison, for HIV-negative patients, the median CD4 counts were 758.5 cells/ml and viral load was log 0/ml. Between HIV+TBPCR+ (n=57) and

HIV+TBPCR- (n=56) categories, the median CD4 count of 321 vs 480 cells/ml (p=0.03), median CD8 count of 480 vs 686 cells/ml (p=0.05), and HIV-1 viral load of log 1.1 vs 0.30 (p<0.001) were higher in HIV+TBPCR+ category. However, between HIV-TBPCR+ and HIV-TBPCR- categories, variables including the median CD4 count of 750 vs 770 cells/ml (p=0.25) and the median CD8 count of 633 vs 690 cells/ml (p=0.28) were not statistically different.

The performance of both MTB confirmatory tests, TBPCR and LJ culture ('gold standard'), was not statistically different between all four groups. Regression analysis revealed a strong correlation between HIV infection status and TBPCR result even after adjusting for CD4 (F=44.97; p=0.02), adjusting for viral load (F=18.18; p=0.05), and when adjusting for both CD4 and viral load (F=31.02; p=0.009).

Conclusions: Incident MTB occurred in HIV-positive patients in Mumbai at median CD4 count of 321 cells/ml and viral load of log 1.1 particles/ml. With new WHO guidelines of 2013 recommending initiation of ART in HIV-positive patients at CD4 count <500 cells/ml, it may also help reduce occurrence of incident cases of MTB substantially.

Introduction:

The World Health Organization (WHO) estimates that over 2 billion individuals globally are infected with Mycobacterium Tuberculosis (MTB) (1). While MTB continues to be a major cause of morbidity and mortality in developing countries, there has been resurgence even in developed countries where it was earlier successfully contained (2). The situation is further compounded by emergence of multidrug resistant (MDR), extreme drug resistant(XDR), and total drug resistant (TDR) MTB (3,4). With the advent of the HIV/AIDS pandemic and its consequent immune suppression, the co-infection of TB/HIV is now recognized as a lethal combination (5). Such co-infection with MTB generally occurred in at least 60-70% of HIV-infected individuals during their lifetime in pre-ART era, often leading to their rapid HIV-disease progression and death (1, 5).

WHO updated the guidelines for initiation of anti-retroviral treatment (ART) for developing countries in 2013 that indicate the CD4 count cut off of <500 cells/ml based on therapeutic benefits against HIV-1 (6). However, these guidelines will also have an added impact on suppressing activation of tuberculosis in HIV-infected persons since there is evidence from studies in Africa that most tuberculosis in HIV-infected persons occurs when their CD4 cell count falls below 350 cells/ml. Due to paucity of evidence on the level of CD4 that trigger incident cases of MTB in HIV-infected patients in Mumbai, a dataset of a study conducted in Mumbai was analyzed to determine the level of CD4 cells and HIV-1 viral count in HIV-infected persons with incident tuberculosis.

Patients &Methods:

Exclusion from IRB Review:

This work is a secondary analysis of data originally collected with informed consent of the subjects; the original study was conducted to determine the sensitivity of a new diagnostic test for tuberculosis. All identifiers designated by the Emory IRB have been removed by the original principal investigator. Therefore, this thesis project does not meet the HHS Office for Human Research Protections (OHRP) definition for "Human Subjects Research" and therefore does not require IRB review. This determination is consistent with the following OHRP criterion (bold indicates applicable portion in this case):

"Research, involving the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens, if these sources are publicly available or **if the information is recorded by the investigator in such a manner that subjects cannot be identified, directly or through identifiers linked to the subjects."**

Subject Population:

The database used for this study was from a prospective case-control study that was conducted at the teaching hospital attached to the MGM University of Health Sciences, Mumbai during pre-ART era (2003-2004) to validate sensitivity/specificity of TBPCR test on peripheral blood against the use of LJ culture as the "gold standard". Assuming that 30% of HIV-positive persons will also be TBPCR+, the sample estimate using EpiInfo 6.03d was taken (HIV-positive case: HIV-negative control) as 4:1. Among the regular outpatient attendees at the Department of Infectious Diseases, a purposive sample

of 113 HIV-positive and 32HIV-negative individuals presenting with clinical features suggestive of incident presumptive or suggestive diagnosis of MTB was recruited for that study. It comprised of adult, non-pregnant individuals who were naïve to anti-tuberculosis treatment (ATT) and ART.

Since the database was used for retrospective analysis of a prospectively collected data, there were no study patient appointments for this analysis. Patients were not contacted for any more information. All information needed for the study existed in the data base but it did not have the patient identifiers (i.e., name, address, city postal codes, dates of hospital attendances and testing/reports, contact numbers).

Laboratory Methods:

All patients were tested for CD4 counts using flowcytometer (Guava technologies, USA) and HIV-1 viral load using RT-PCR (Amplicor, Roche, USA) at the time of recruitment. Five milliliters of peripheral blood was collected from study participants in EDTA vacutainers (Becton Dickinson, USA) after obtaining the individual informed written consent. LJ medium slants (Hi-Media laboratory, Mumbai) were inoculated with 0.2 ml of sediment from isolator lysis tubes (7). The remaining sample was processed for TB-PCR targeted to insertion sequence (IS) 1081 as per the detailed protocol published in an earlier study (8).

Primary Study Objective:

To determine the level of CD4 and HIV-1 viral count in HIV-infected persons in Mumbai, India developing incident MTB.

Analysis Plan:

The primary endpoints for this analysis were incident cases of tuberculosis, because all patients were naïve to anti-tuberculosis treatment and ART. The "exposure" variables of interest were CD4 count and HIV-1 viral load. Based on literature review, data that were included in the analysis as potential confounders were: a) Demographic information such as age, gender (5); b) Clinical/Laboratory information such as severe weight loss, recurrent fever, chronic cough, abdominal lump suggestive of MTB, neck stiffness suggestive of MTB meningitis (12), sputum for AFB staining/LJ Culture/TBPCR, CSF for AFB/LJ culture/TBPCR, pleural fluid for AFB/LJ culture/ TBPCR, X-ray chest, full blood counts and ESR, and tuberculin skin test (8).

Data were analyzed using EpiInfo 6.03 (CDC Atlanta). A bivariate analysis was performed to determine associations between the endpoint of interest (occurrence of incident MTB) and the exposure variable, namely the CD4 count and HIV-1 viral load.

A multivariate analysis was performed to determine the independent association between CD4/HIV-1 viral counts and incident MTB after adjusting for all variables found to be significant at $p<0.05$ in the bivariate analysis or thought to be clinically relevant

regardless of statistical significance i.e. ESR, TST, x-ray chest, TBPCR, CD4, CD8 and HIV-1 viral load.

The statistical significance of categorical and continuous variables were assessed by the X^2 and students 't' test, respectively. Analysis for test of significance using $X^2$was done to validate the blood TBPCR against the LJ culture; latter was used as the 'gold standard'.

Ethical considerations:

Before giving the database, the PI of the study had removed all patient identifiers such as the name, address, postal codes, hospital registration number, dates of hospital attendances and diagnostic tests/reports, mobile numbers, etc. No new information was collected from patients in the cohort. All information was stored in a password protected database. Because of the nature of this analysis, no application to IRB of Emory University was required. Also, the retrospective design of this study did not require re-consent of subjects.

Results:

The study cohort comprised of 145 patients; 113 were HIV-positive and 32 were HIV-negative. Although all 145 patients had clinical features suggestive of MTB, 74 were confirmed to have MTB using TBPCR and LJ culture tests. The mean age of the cohort was 33.5 $\pm$9.9 years. The male: female ratio was 4:1 that was similar to the general profile of attendees at the outpatient clinic of ID department. The mean age of male

patients was not statistically different ($p>0.05$) at 34.4 $\pm$ 10.0 as compared with 30.60 $\pm$ 9.3 years for female patients.

Among 113 HIV-positive patients, the median CD4 count at recruitment with TB diagnosis was 340 cells/ml and viral load was log 0.6/ml. By comparison, for HIV-negative patients, the median CD4 counts were 758.5 cells/ml and viral load was log 0/ml (table 1).

The multivariate analysis to determine factors associated with HIV and TBPCR status of patients in four categories, namely HIV+TBPCR+, HIV+TBPCR-, HIV-TBPCR+, and HIV-TBPCR- is presented in table 2. Mean age, gender distribution, clinical staging of HIV/AIDS and clinical features (**weight loss, fever, cough, lump in abdomen, neck stiffness)** were not significantly different within the HIV-positive and HIV-negative categories. On comparing between HIV+TBPCR+ (n=57) and HIV+TBPCR- (n=56) categories, the median ESR of 74 vs 50 mm ($p=0.15$), median tuberculin skin test (TST) of 16 vs 8 mm ($p=0.15$) and MTB suggestive changes in chest x-rays ($p<0.001$) were higher in HIV+TBPCR+ category. Also, in HIV+TBPCR categories, the median CD4 count of 321 vs 480 cells/ml ($p=0.03$), median CD8 count of 480 vs 686 cells/ml ($p=0.05$), and HIV-1 viral load of log 1.1 vs 0.30 ($p<0.001$) were significantly higher.

However, between HIV-TBPCR+ and HIV-TBPCR- categories, no variable was statistically different, including the median CD4 count of 750 vs 770 cells/ml ($p=0.25$) and the median CD8 count of 633 vs 690 cells/ml ($p=0.28$). This suggests that MTB

activation in HIV-negative patients, contrary to HIV-positive patients, do not seem to correlate with level of CD4 or CD8 counts (table 2).

Since there was no statistically significant difference between the two MTB confirmatory tests, namely the TBPCR and the LJ culture ('gold standard'), there was general agreement of TBPCR test results with those of LJ culture test (tables 3-6).

Regression analysis revealed a strong correlation between HIV and CD4 (F=82.55), HIV and viral load (F=33.16), TBPCR and ESR (F=13.27) and TBPCR and TST (F=67.20) (table 7). There was a strong correlation between HIV and TBPCR even after adjusting for CD4 (F=44.97), adjusting for viral load (F=18.18), and when adjusting for both CD4 and viral load (F=31.02).

Discussion:

Before the advent of anti-retroviral treatment in developing countries, 60-70% of HIV-infected individuals developed active clinical MTB during their lifetime (1,5). With activation of MTB, the HIV disease progression was accelerated; hence TB-HIV was considered a lethal combination (5). The study underscores the importance of quick diagnosis of TB using PCR assay with primers such as those with IS1081 that are prevalent in India (9). The study has established the value of early diagnosis of TB from peripheral blood at an early point of activation when most patients did not produce other traditional specimens such as the sputum and/or pleural fluid. In fact, only 31/74 (41.9%)

of TBPCR positive patients had produced specimens such as sputum and/or pleural fluid at the time of their diagnosis. In other words, over 60% of early TB infection in this cohort would have been missed if the peripheral blood was not tested with the PCR assay and if treating physician waited for the patients to produce sputum and/or pleural fluid. Early diagnosis of MTB was followed with prompt treatment, and for co-infected patients, ART was offered. Thus, the value of early diagnosis of TB using PCR cannot be overemphasized.

In this study, there was agreement between TBPCR and LJ culture results. Our earlier study using the same primer IS1081 established a sensitivity and specificity for TBPCR of 83% and 97%, respectively (8). Following prompt rifampicin-containing anti-MTB treatment, patients showed clinical improvement such as weight gain, disappearance of cough and fever within weeks of initiation of treatment and no deaths occurred. The average time for PCR conversion to negative after treatment was 6-8 weeks (8). Thus, even in cases of mild tuberculosis infection, PCR is a sensitive assay compared to LJ medium culture, the latter is likely to fail or take longer time (4-8 weeks) to produce a positive result with respect to this slow-growing organism. Various factors such as immune competence, time required for clearance of bacilli from the blood and other factors may differ from individual to individual; hence there is a difference in the time required for PCR conversion. Our earlier study showed that both live and dead bacilli were cleared from blood in about 6-8 weeks of anti-MTB treatment (8). This is an important finding that clinicians can use to determine the therapeutic response or the emergence of resistance (MDR/XDR TB) during treatment. Considering that TBPCR

using easily accessible peripheral blood sample has reportedly high sensitivity and specificity and has results available within 48 hours, it should be included within the WHO algorithm for the management of HIV/TB co-infections. This recommendation is feasible, even for the developing countries because WHO and many international agencies are assisting with installation of PCR facilities, primarily for 'Early Infant Diagnosis for HIV'. TBPCR can be clubbed-on with this PCR facility to strengthen HIV/MTB co-infection program. The Xpert MTB/RIF test endorsed by WHO is a rapid test that uses liquid culture medium in a dedicated instrument and needs sputum/pleural fluid specimens for testing (10,11). Considering that <40% of HIV/MTB co-infected patients produce sputum due to non-productive cough attributed to poor pulmonary phagocytic activity and tissue necrosis, the TBPCR test used in this study using IS 1081 sequence is an ideal alternative for the remaining 60% of co-infected patients who do not produce sputum.

The clinical presentation of co-infected patients in decreasing order of frequency was fever, severe weight loss, cough, and abdominal lumps (table 2). This pattern was similar to that published earlier (12).

Several important diagnostic markers for MTB were established in this study. It revealed that chest X-ray was significantly suggestive of MTB, both in HIV+TBPCR+ and HIV-TBPCR+ patients (table 2). Also, regression analysis showed significant correlation of HIV with CD4 and viral load and also significant correlation of MTB with ESR and TST tests (table 7). An earlier study in Spain showed that TST-positive patients were at high

risk of developing TB, irrespective of their CD4 count (13). However, among TST-negative patients only those with a CD4 count < 200 cells/μl have an appreciable risk of developing the disease. The re-emergence of these important tests in this study not only validates the quality of this study but also their public health implications. All, chest x-ray, ESR, and TST are cost-effective tests for complementing the clinical diagnosis of MTB but are now rarely used due to availability of sophisticated newer technologies. The high correlation of ESR (F=13.27) and TST (F=67.20) with the diagnosis of MTB should be re-deployed in the clinical settings.

The occurrence of incident MTB among HIV-positive patients in this cohort at median CD4 count of 321 cells/ml and median viral load of log 1.1 particles/ml confirms the findings of other studies in Africa and elsewhere (14-16). In individuals with latent MTB infection, CD4 depletion accelerates the progression from latent infection to active tuberculosis, which, in turn, is believed to further fuel HIV replication rates due to elevated levels of pro-inflammatory cytokines [17]. A recent, retrospective study from Italy showed an impaired immune recovery in HIV/TB cases compared to AIDS caused by other co morbidities which seemed not to recover even after 3 years and despite access to efficient ART (18). However, the HIV patients without MTB when put on ART within the first 2 months or from 2-5 months, showed an increase in CD4 counts of 69 (95% CI: 22; 117) and 110 (95% CI: 52; 168), respectively (18). By comparison, HIV-negative patients who developed incident MTB had a median CD4 count of 750 cells/ml in this study. This observation is contrary to two other studies that suggest that MTB by itself is associated with transitory lymphopenia including the CD4 positive cells (19,20). This

could either be explained by continued sequestering of cells to the lungs or due to apoptosis and persistent regulatory stimuli [21-23].

A study of a large prospective cohort of newly diagnosed pulmonary MTB patients in Tanzania reported the CD4 lymphocyte dynamics during TB treatment; both in HIV-uninfected and HIV-infected TB patients who were ART-naïve (24). Their mean CD4 count of 285 cells/μL (95% CI 269;301) among the HIV-infected MTB patients is in conformity with our study findings. Similarly, in a Ugandan study, the HIV-infected MTB patients who were put on ART at the time of MTB diagnosis did not increase their pool of circulating CD4 cells during the 5 months observation and treatment period (25).Mortality of HIV/TB patients in Eastern Europe was three- to-nine fold higher than in Western Europe. MTB, despite treatment was the main cause of death in Eastern Europe in 80%, 66% and 61% of patients who died, 3 months, 3–12 months or 12 months after TB diagnosis, compared to 50%, 0% and 15% in the same time periods in Western Europe (p=0.0001). Although performance of TB programs in Eastern and Western Europe differ considerably, this finding has important implications for HIV/TB programmes to aim at averting incident MTB so as to limit TB-associated mortality (26).

The study analysis had several limitations. Being a dataset of a purposive sample, selection bias of investigators towards recruiting sick patients having features suggestive of MTB could skew causal effect of HIV-1 on CD4 and HIV-viral load test results. Since the data-set dates back to 10 years, the causal relationship of HIV-1 with CD4/CD8 and HIV-1 viral load will depend on pathogenesis of then circulating HIV-1 subtypes in

Mumbai. Also, recombinant circulating forms emerging over the past decade could now have altered pathogenesis of circulating subtype of HIV-1 C3, thus limiting operational applicability of this data.

In view of our study findings, the new WHO guidelines of 2013 recommending initiation of ART at CD4 cell count <500/ml appear appropriate because ART will also help to prevent immune deterioration and consequent emergence of incident MTB cases. This will not only reduce high mortality attributable to MTB but also will avert MTB-induced CD4 damage that is reported not to recover after successful anti-MTB treatment and even after 3 years of successful ART (18). Certainly, the WHO guidelines of 2010 for initiation of ART at CD4 count of <350 cells/ml will fall short in averting incident cases of MTB in HIV-positive patients (16). The wider public health benefit of WHO 2013 strategy cannot be overemphasized because aversion of new incident cases of MTB is an added benefit that will also reduce secondary transmission of MTB in the community.

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TABLE 1: UNIVARIATE ANALYSIS OF CD4/CD8 AND HIV-1 VIRAL LOAD AMONG 145 HIV-POSITIVE AND HIV-NEGATIVE PATIENTS IN A PROSPECTIVE STUDY IN MUMBAI, 2003-2004.

	HIV-positive	HIV-negative
CD4 • Mean • Median • T-stat • Two tailed P-Value	 397.5 +/-sd 207.5 340.0 +/- IQR 20.36 0.03	 738.4 +/- sd 80.0 758.5 52.17 0.01
CD8 • Mean • Median • T-stat • Two tailed P-Value	 623.8 +/- sd 305.5 612 21.71 0.02	 649.7 +/- sd 590.9 669.0 62.20 0.01
Viral Load • Mean • Median • T-stat • Two tailed P-Value	 Log 0.94 +/_ sd 0.92 Log 0.60 10.85 0.06	 0 0 0 N/A

TABLE 2: DESCRIPTIVE ANALYSIS OF FACTORS ASSOCIATED WITH HIV AND TBPCR STATUS OF 145 PERSONS ENROLLED IN A PROSPECTIVE STUDY, MUMBAI, 2003-2004.

	HIV+TBPCR+ n=57 (%)	HIV+TBPCR-n=56 (%)	p-value for HIV+TBPCR+ / HIV+TBPCR- comparison	HIV-TBPCR+ n=17 (%)	HIV-TBPCR- n=15 (%)
Mean age (yrs)	**34.9 +/- sd 7.3**	**35.4 +/- sd 8.4**	**NS**	**31.1+/-sd 18.2**	**23.8 +/- sd 4.0**
Gender • **Male** • **Female**	 **44 (77.2)** **13 (22.8)**	 **46 (82.1)** **10 (17.9)**	**NS**	 **13 (76.5)** **4 (23.5)**	 **9 (60.0)** **6 (40.0)**
Clinical Stage • **AIDS** • **ARC** • **Asymp** • **HIV-negative**	 **20 (35.1)** **27 (47.4)** **10 (17.5)** **N/A**	 **19 (33.9)** **22 (39.3)** **15 (26.8)** **N/A**	**NS**	 N/A N/A N/A 17	 N/A N/A N/A 15
Clinical Features • **Wt loss** • **Fever** • **Cough** • **Lump abd** • **Neck stiffness**	 **27 (47.4)** **34 (59.6)** **22 (38.6)** **5 (8.8)** **0**	 28 (50.0) 24 (42.9) 24 (42.9) 0 0	**NS**	 **10 (58.8)** **14 (82.4)** **12 (70.6)** **2 (11.8)** **3 (17.6)**	 **2 (13.3)** **2 (13.3)** **3 (20.0)** **0** **0**
Past Anti-TB treatment	**0**	**0**		**0**	**0**
Lab specimens • **Sputum** • **CSF** • **Blood** • **Pleural fluid**	16 --- 16AFB+* 0 57 1-----1 AFB+	**17----3** **AFB+*** **1----1** **AFB+*** **56** **0**	**NS**	**10 --9** **AFB+*** **3 --2 AFB** **+*** **17** **4---** **3AFB+**	**0** **0** **15** **1-------** **1AFB+**
ESR • **Mean** • **Median**	**69.7 +/- sd 40.9** **74.0**	**56.1+/-sd 36.2** **50.0**	**MH X2=2.03 OR=1.45(0.84, 2.51) P=0.15**	**77.5+/-sd42.9** **67.0**	**16.5+/-sd19.5** **10.0**
Skin Tuberculin Test	**15.5+/-**	**9.4+/-sd5.1**	**MH X2=2.08 OR=1.96(0.72,**	**17.8+/-5.9**	**7.5+/-sd2.9**

• **Mean size** • **Median size**	**sd5.4** **16.0 mm**	**8.0 mm**	**5.49)** **P=0.15**	**18.0 mm**	**6.0 mm**
X-ray chest suggestive of TB	**35 (61.4)**	**10 (17.9)**	**MH X2=9.82** **OR=3.44(1.46, 8.24)** **P=0.001**	**14 (82.4)**	**0**
Acid Fast Bacilli • **Positive** • **Negative** • **No specimen**	**17 (33.3)** **3 (5.3)** **35 (61.4)**	**4 (7.1)** **19 (33.9)** **33 (58.9)**	**X2=21.47** **P<0.001**	14(70.6) 3 (17.6) 2 (11.8)	**0** **0** **15**
Provisional Dx of TB • **Presumptive** • **Suggestive** • **Latent/Neg**	**24 (42.1)** **26 (45.6)** **7 (12.3)**	**4 (7.1)** **19 (33.9)** **33 (58.9)**	**NS**	**15 (88.2)** **2 (11.8)** **0**	**1 (6.7)** **1 (6.7)** **13 (86.6)**
LJ culture positive	**54**	**2**	**NS**	**14**	**1**
CD4 count • **Mean** • **Median**	**337.0+/-168.4** **321.0**	**459.1+/-226.1** **480.0**	**MH X2=4.38** **OR=1.52(1.01, 2.30)** **P=0.03**	**726.5+/-88.2** **750.0***	**751.9+/-70.2** **770.0***
CD8 count • **Mean** • **Median**	**567.0+/-312.7** **480.0**	**681.7+/-289.3** **686.0**	**MH X2=3.64** **OR=1.45(0.91, 2.18)** **P=0.05**	**624.5+/-52.6** **633.0***	**678.3+/-54.1** **690.0***
HIV-1 viral load • **Mean log** • **Median log**	**1.21+/-0.91** **1.1**	**0.66+/-0.85** **0.30**	**MH X2=53.02** **OR=3.60(2.45, 5.29)** **P<0.001**	**0** **0**	**0** **0**

*Represent number of specimens available for testing/number specimens that tested Acid Fast Bacilli (AFB) positive.
Not Significant (NS) statistics

TABLE 3: HIV STATUS BY PROVISIONAL CLINICAL DIAGNOSIS OF TUBERCULOSIS AMONG 145 PERSONS ENROLLED IN A PROSPECTIVE STUDY, MUMBAI, 2003-2004.

HIV status	Presumptive TB	Suggestive TB	No TB/Latent TB	Total
Positive	31	64	18	113
Negative	16	03	13	32
Total	47	67	31	145

TABLE 4: PROPORTION OF HIV-POSITIVE AND HIV-NEGATIVE PATIENTS, WITH AND WITHOUT TUBERCULOSIS, WHO HAD A POSITIVE TBPCR AND/OR LJ CULTURE TEST, WITH A PROVISIONAL DIAGNOSIS OF TUBERCULOSIS*.

Provisional Dx	TBPCR+	LJ culture +	TBPCR-ve	LJ culture - ve	Total
Presumptive	39	37	08	7	47
Suggestive	28	27	39	38	67
No TB/Latent TB	07	03	24	23	31
Total	74	67	71	68	145

*X^2=31.53; p=0.000

TABLE 5: PROPORTION OF POSITIVES USING TBPCR AND LJ CULTURE TESTS, IN PATIENTS WITH A PROVISIONAL DIAGNOSIS OF TUBERCULOSIS*.

Provisional Dx	TBPCR+	LJ culture +
Presumptive	39	37
Suggestive	28	27
No TB/Latent TB	07	03
Total	74	67

*X^2=1.33; p0.51

TABLE 6: PROPORTION OF NEGATIVES USING TBPCR AND LJ CULTURE TESTS, IN PATIENTS WITH A PROVISIONAL DIAGNOSIS OF TUBERCULOSIS*.

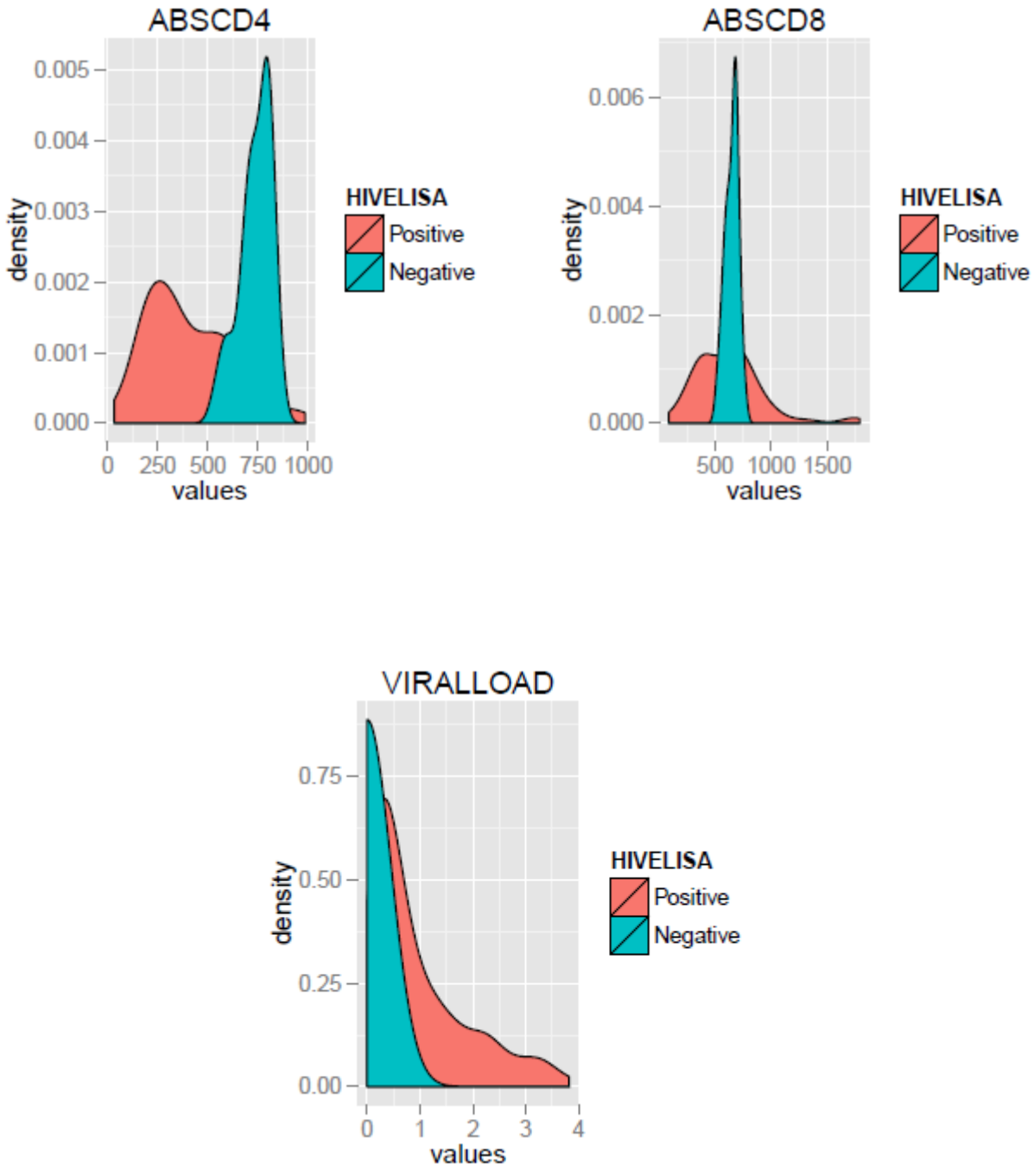
Provisional Dx	TBPCR-ve	LJ culture -ve
Presumptive	8	7
Suggestive	39	38
No TB/Latent TB	24	23
Total	71	68

*X^2=0.14; p=0.93

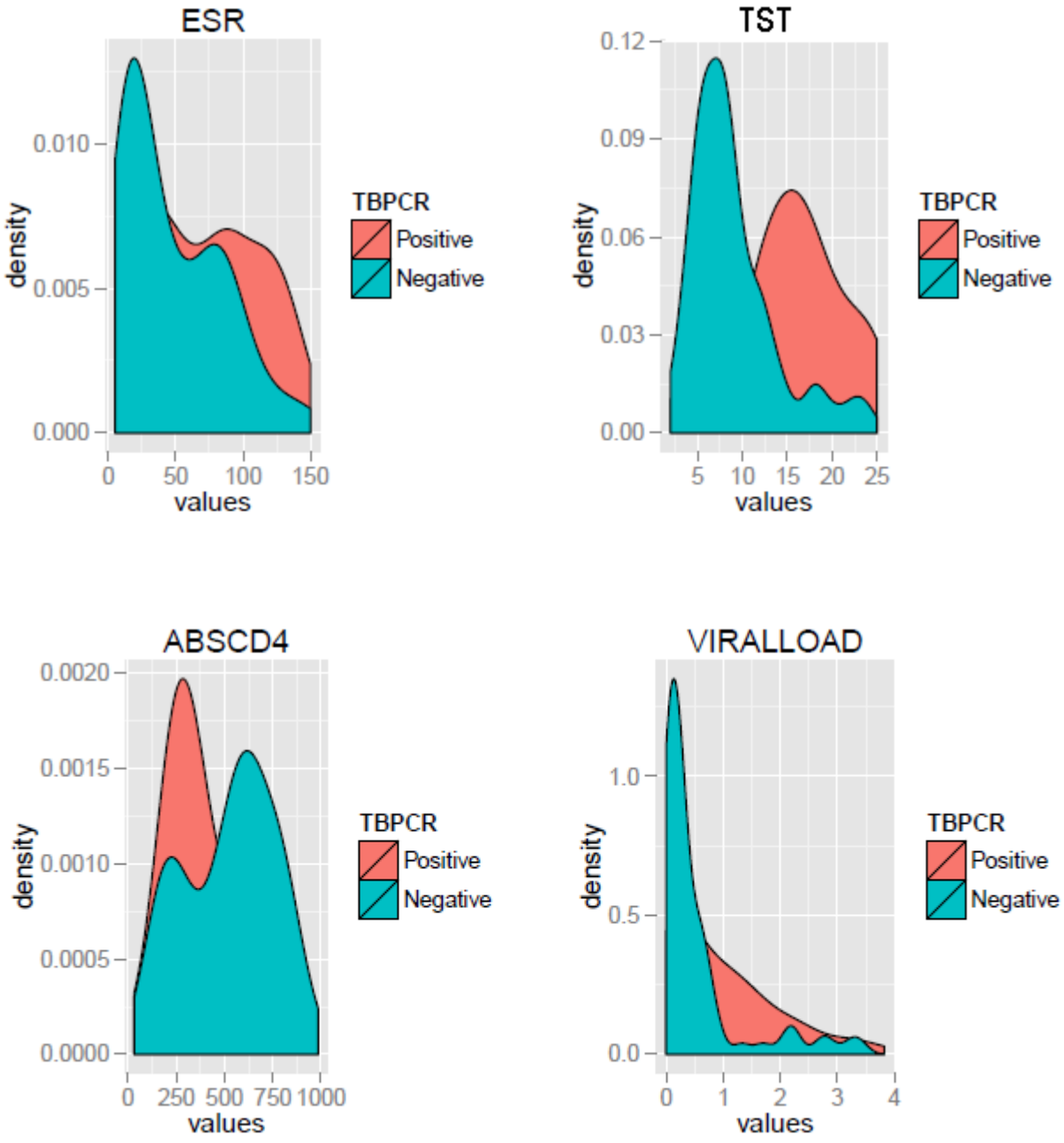
TABLE 7: MULTIVARIATE REGRESSION ANALYSIS OF CONFOUNDING VARIABLES AMONG 145 PERSONS IN A PROSPECTIVE HIV/TUBERCULOSIS STUDY, MUMBAI, 2003-2004.

Variables	Df(d)	Correlation Coefficient	F-statistics (p-value)
HIVELISA + TBPCR	1	r^2=0.00	0.07
HIVELISA + ESR	1	r^2=0.00; ra^2=0.09	7.98
HIVELISA + TST	1	r^2=0.00	0.13
HIVELISA + CD4	1	r^2=0.37 (CI 0.22,0.50)	82.55(0.08)
HIVELISA + VL	1	r^2=0.19 (CI 0.16,0.50)	33.16(0.12)
TBPCR + ESR	1	r^2=0.08 (CI -0.28,0.49)	13.27(0.19)
TBPCR + TST	1	r^2=0.32;(CI -0.41,0.68)	67.20(0.09)
TBPCR + CD4	1	r^2=0.04	6.09
TBPCR + VL	1	r^2=0.05	7.90
HIVELISA + TBPCR + age	2	r^2=0.10; ra^2=0.09	7.8
HIVELISA + TBPCR + gender	2	r^2=0.01; ra^2=-0.00	0.87
HIVELISA + TBPCR + ESR	2	r^2=0.02; ra^2=0.01	1.81
HIVELISA + TBPCR + TST	2	r^2=0.00; ra^2=-0.01	0.07
HIVELISA + TBPCR + CD4	2	r^2=0.39; ra^2=0.38	44.97 (0.02)
HIVELISA + TBPCR + CD8	2	r^2=0.00; ra^2=-0.01	0.18
HIVELISA + TBPCR + VL	2	r^2=0.20; ra^2=0.19	18.18(0 .05)
HIVELISA + TBPCR + age + gender	3	r^2=0.10; ra^2=0.09	5.47
HIVELISA + TBPCR + ESR + TST	3	r^2=0.03; ra^2=0.01	1.34
HIVELISA + TBPCR + CD4 + VL	3	r^2=0.40; ra^2=0.38	31.02(0 .009)

PLOT 1: HISTOGRAMS SHOWING DENSITY OF ABSOLUTE CD4, ABSOLUTE CD8, AND HIV-1 VIRAL LOAD IN HIV-POSITIVE/HIV-NEGATIVE PERSONS.



PLOT 2: HISTOGRAMS SHOWING DENSITY OF ESR, TST, ABSOLUTE CD4, AND HIV-1 VIRAL LOAD IN TBPCR+/TBPCR- PERSONS.



CHAPTER III: SUMMARY OF CONCLUSIONS/PUBLIC HEALTH IMPLICATIONS OF THE STUDY

Studies conducted in the pre-ART era in India had established that 8.3% of HIV-infected persons developed active clinical MTB each year, generally when CD4 counts fell<300 cells/ml (5,12). Our study re-affirms that incident MTB occurred in HIV-positive patients at median CD4 count of 321 cells/ml and viral load of log 1.1 particles/ml. In view of our study findings, the new WHO guidelines 2013 recommending initiation of ART in HIV-positive patients at CD4 count <500 cells/ml appear appropriate since ART will also help reduce occurrence of incident cases of MTB in India substantially.

The value of CD4 and HIV-1 viral load as important markers in HIV-positive patients was established in the study once again. Since CD4 test is not predictive of incident MTB occurrence in HIV-negative patients, it seems to be of no value in those patients. However, for the diagnosis of MTB in both HIV-positive and HIV-negative patients, the following tests were significantly predictive: chest x-ray, ESR, TST, and TBPCR.

TBPCR test using primers with Insertion Sequence 1081 has a high sensitivity and specificity for the diagnosis of MTB in India. Its results have good agreement with those of LJ culture test. Periodic screening of HIV-positive patients is facilitated because it uses peripheral blood that is easy to obtain by venepuncture. This recommendation is feasible, even for the developing countries because WHO and many international agencies are assisting with installation of PCR facilities, primarily for ‘Early Infant Diagnosis for HIV’. TBPCR can be clubbed-on with this PCR facility to strengthen HIV/MTB co-

infection program. TBPCR assay costs between $15-20 in India. It can be rapidly deployed in major cities with high burden of TB because of lower cost for the local population, large hospitals that have PCR infrastructures, and trained manpower which is able to perform quality PCR testing. The risk of over diagnosis of TB using peripheral blood can be reduced by instituting a good quality assurance program and linking PCR diagnostic laboratories to research institutes.