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Diagnosis of Latent Tuberculosis Infection among HIV discordant partners using Interferon-γ Release Assays (IGRAs).

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An abstract of
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in partial fulfillment of the requirements for the degree of Master of Science in Clinical Research

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ABSTRACT

There is limited data on the effect of HIV status and CD4 counts on performance of Interferon-γ Release assays (IGRAs) for diagnosis of latent tuberculosis infection (LTBI).

A cross sectional study was conducted to assess the prevalence of and risk factors for a positive diagnostic test for LTBI, using tuberculin skin test (TST) and IGRAs among HIV-discordant couples in Zambia.

A total of 596 subjects (298couples) were enrolled. Median CD4 count among HIV positive persons was 388 cells/ µl, (range 51-1330). HIV negative persons were more likely than their HIV positive partner, to have a positive diagnostic test for LTBI with TST (203 vs 128), QFT (171 vs 109) and TSPOT (156 vs. 109). On multivariate analysis, HIV negative status was an independent predictor for a positive QFT (OR=2.22, 95% CI 1.42- 3.46) and TSPOT (OR=1.79, 95% CI 1.16-2.77). Among HIV positive subjects a CD4 count ≥388cells/µl was associated with a positive TST (OR= 1.76 95% CI 1.10-2.82) and QFT (OR=1.71 95% CI 1.06-2.77) but not TSPOT (OR =1.20 95% CI 0.74-1.94).

Persons with HIV had significantly fewer positive diagnostic tests for LTBI with TST, QFT and TSPOT. Persons with a CD4 count <388 cells/µl were less likely to have a positive TST or QFT, but not less likely to have a positive TSPOT. TSPOT may perform better than TST or QFT in HIV positive individuals.

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INTRODUCTION

HIV and tuberculosis (TB) are the leading causes of death among adults due to an infectious disease worldwide. It is estimated that >13 million people are co-infected with HIV and *Mycobacterium tuberculosis* [1]. If a person is exposed to the *M. tuberculosis* bacteria but does not develop active disease, they are said to have latent tuberculosis infection (LTBI). Persons with LTBI have a 10% lifetime risk of developing active TB. Persons with HIV have a 10% per year risk of developing active TB disease [2-4].

A major strategy to control the spread of active TB is the diagnosis and treatment of latent tuberculosis infection [5-8]. The WHO recommends use of isoniazid prophylaxis in persons with LTBI to prevent active TB [6]. Until recently diagnosis of LTBI relied on the Tuberculin Skin Test (TST), a test that has many limitations, including poor sensitivity and specificity [2]. A new generation of diagnostic tests is now available called Interferon Gamma Release Assays, that rely on release of interferon-gamma from T cells, in response to TB specific antigens. While there is some data to suggest these tests work well in immunocompetent persons, there is limited data on patients with HIV.

The purpose of our study is to evaluate the performance of Interferon Gamma Release Assays in HIV positive persons and compare results to a control population of HIV negative subjects. The study was conducted at the Zambia Emory HIV Research Project which is a prospective cohort of HIV discordant couples. We enrolled 596 subjects, 298 HIV positive and 298 that were HIV negative. The results of this study are expected to help determine whether Interferon Gamma Release Assays provide any advantage over the traditional TST in the diagnosis of LTBI in HIV positive individuals.

BACKGROUND

HIV and tuberculosis (TB) are the leading causes of death among adults due to an infectious disease worldwide. It is estimated that >13 million people are co-infected with HIV and *Mycobacterium tuberculosis* [1]. The World Health Organization (WHO) estimates that there are approximately 9.3 million new cases of active TB and nearly 2 million deaths due to the disease worldwide each year [7,8]. Twenty-seven percent of TB cases and 31% of TB-related deaths occur in Africa, home to only 11% of the world's population [4, 6].

Latent tuberculosis infection (LTBI) is when a person is exposed to *M. tuberculosis* bacteria, but does not develop active disease. HIV infection is the most important risk factor for progression from latent tuberculosis infection to active TB [2,3]. In patients with HIV and LTBI, the annual risk of progression to active TB is approximately 10% per year compared to a lifetime risk of 5-10% in immunocompetent persons [2-4]. Diagnosis and treatment of LTBI is a major strategy for TB control and prevention in the US [9, 10]. WHO has recommended the implementation of isoniazid preventive therapy for HIV-seropositive persons in an effort to prevent additional cases of TB, but this strategy has not yet been widely adopted in Africa [8].

For nearly a century, diagnosis of LTBI has relied on the tuberculin skin test (TST) which has several limitations. The test is subjective and requires the patient to return for a second visit at 48-72 hours. In addition, there is low specificity due to cross reaction with BCG vaccination and non-tuberculous mycobacteria (NTM) , and low sensitivity in HIV

infection. New diagnostic tests for tuberculosis are urgently needed to enhance global TB control [11, 12].

A new generation of tests called Interferon-γ release assays (IGRAs) are now commercially available for the diagnosis of LTBI (QuantiFERON-TB Gold in Tube test [QFT] and T-SPOT.TB [TSPOT]) [13,14]. These tests rely on the release of Interferon-gamma from sensitized T cells in response to TB specific antigens ESAT6 and CFP10. A patient's blood is mixed with TB specific antigens, if the patient has been exposed to TB in the past, his effector T cells will recognize the antigens and secrete interferon-gamma. This can be measured using an ELISA in the QFT test, and using an ELISPOT reader in the TSPOT test. The crucial difference between these tests is the QFT uses 1 ml of a patient's blood, while the TSPOT test uses 250,000 mononuclear cells counted from the patient's blood to perform the assay.

IGRAs provide increased specificity over TST, because the antigens used in the test do not cross react with BCG or other NTM. However, there is limited data on whether these tests provide any benefit over TST in immunocompromised individuals, such as those with HIV, particularly in high prevalence countries [14-21].Studies that have been published to date have often used 1st generation tests, have small sample size, have no CD4 count data and did not compare TST, QFT and TSPOT in the same patient population. In addition most studies lacked an HIV negative control group for comparison.

We conducted an observational cohort study to assess the performance of three diagnostic tests for LTBI (TST, QFT and TSPOT) in HIV positive persons and compared these results to the results of their HIV negative partner (control group), in Zambia. The goals of our study were: 1. To assess prevalence of a positive test for latent tuberculosis infection; 2. To assess concordance between TST, QFT and IGRAs; 3. To determine whether HIV is a risk factor for a positive test with TST, QFT or TSPOT; 4. Among HIV positive individuals to determine if CD4 count is an independent predictor for a positive TST, QFT or TSPOT.

METHODS

The purpose of this study was to assess whether interferon gamma release assays perform as well in diagnosing LTBI in HIV positive as they do in HIV negative persons.

Null Hypothesis:

The proportion of positive test results with TST, QFT or TSPOT in persons with HIV is statistically equal to the proportion of positive test results with TST, QFT or TSPOT in HIV negative individuals.

Alternative Hypothesis:

The proportion of positive test results with TST, QFT or TSPOT is statistically different when comparing HIV positive to HIV negative individuals.

Study Design:

The study was a cross sectional observational cohort.

Study Site:

The study was conducted in Lusaka at the Zambia Emory HIV Research Project (ZEHRP). ZEHRP consists of a cohort of heterosexual HIV discordant couples and promotes couple's voluntary counseling and testing (CVCT) as a method of HIV prevention and as an entry-point into HIV clinical care [22]. Recruitment and study procedures have been described elsewhere [23- 27].

Inclusion Criteria:

HIV-infected individuals (>16 years of age) and their discordant (HIV-seronegative) partner were offered an opportunity to enroll in the study. Subjects had to come to clinic together and had to be age >16 years.

Exclusion criteria:

Current pregnancy, active TB, or if the couple failed to come to the clinic appointment together were exclusion criteria.

IRB Approval:

The study was approved by the Emory University Institutional Review Board (IRB) and the University of Zambia Research Ethics Committee and study subjects provided written informed consent.

Study procedures:

Each study subject had two study visits. At the first study visit, study subjects were asked to complete a questionnaire that included questions regarding exposure to TB at home, history of BCG vaccination and history of incarceration. Blood was drawn from study participants for IGRA tests (3 ml for the QFT test [Cellestis Inc, Australia] test and 8 ml for the TSPOT [Oxford Immunotec, Oxford, UK]). HIV infected persons had a CD4 count performed. Following the blood draw, a TST was placed using 0.1 ml of PPD reagent (Tubersol, Sanofi Pasteur) by the Mantoux method [6]. Study participants were instructed to return in 48 to 72 hours to have the TST read. The amount of induration (in mm) was recorded by a trained health care provider. Persons with a positive TST had a chest radiograph performed and were questioned about symptoms in order to exclude active TB disease. The QFT and TSPOT tests were performed based on the manufacturer's instructions and as previously reported [12,13,14,16]. Interferon-γ release was measured using ELISA after stimulation of sensitized T-cells using TB specific antigens. For TSPOT 250,000 peripheral blood mononuclear cells (PBMCs) were isolated and plated per well. Each test consisted of four wells: a negative control, a positive control (PHA) and TB specific antigens (CFP10 and ESAT 6). Spot forming

units were counted manually and using an EliSpot Reader, (Cellular Technology Ltd., Cleveland, OH). All reported TSPOT results are based on results from the ELISPOT Reader.

Definition of a positive test result:

Based on national and international guidelines, a positive TST was defined as ≥5 mm of induration in HIV-infected persons and ≥10 mm in HIV-seronegative persons [7]. QFT was positive if the interferon-γ response to TB antigens minus the negative control was ≥0.35 IU/ml and >25% of the negative control; negative if these criteria were not met; and indeterminate if either the negative control had a result of ≥8 IU/ml, or if the positive control had a result of < 0.5 IU/ml of interferon-γ. A positive TSPOT test was defined as a response to either ESAT6 or CFP10 minus the negative control that is ≥ 8 spot forming cells, or >2 times the negative control; the test was negative if these criteria were not met; and the test was indeterminate if the reading in the negative control was > 20 spots or if the reading in the positive control were < 20 spots.

Sample size calculation:

Sample size calculation was performed using prevalence of LTBI in Zambia from a previous study [28]. The precision method was used, by estimating the half width of the 95% confidence intervals we calculated a sample size of 300 couples. **(Appendix A)**

Data Analysis:

Data analysis was performed using SAS 9.1 (SAS Inc, Cary, NC). Outcomes of interest included: 1. Prevalence of a positive test for LTBI using TST, QFT and TSPOT; 2.

Comparison of rates of TST, QFT and TSPOT positivity between HIV positive and HIV negative subjects using chi-square test. 3. Concordance between diagnostic tests, measured using κ-statistic , where $\kappa > 0.75$ is excellent agreement, κ 0.4–0.75 is fair to good agreement and $\kappa < 0.4$ represents poor agreement [13]; 4. Risk factors associated with a positive test result with TST, QFT and TSPOT were measured using univariate and multivariate analysis and prevalence odds ratios and 95% confidence intervals were reported. Risk factors of interest included age, gender, HIV status , CD4 count, income , h/o prison stay, household contact with active TB in the last month and BCG vaccination.

Purposeful selection of covariates for logistic regression model:

The outcome of interest was a positive TST, QFT or TSPOT. The primary exposure of interest was HIV status. Initially univariate descriptive analysis was performed in order to determine risk factors for a positive test. Interaction was evaluated using the Breslow Day test and Wald chi square test. We also assessed for potential confounding by determing whether there was >10% change in the odds ratio for the effect of HIV on a positive test result. Co linearity was looked at using Dan Rosen's SAS macro and goodness of fit was assessed using the Hosmer-Lemeshow test. Variables that were significant on univariate analysis , had biological plausibility or were confounders or effect modifiers were included in the final model.

We used conditional logistic regression to investigate the relationship between HIV status and a positive test result for LTBI. Since we are matching on marriage as a surrogate for similar exposures and socioeconomic status we chose to use this matched design in the

analysis as well. This would result in a large number of parameters relative to the number of observations and therefore unconditional logistic regression would result in a biased result. Unconditional logistic regression was used among HIV positive persons to evaluate the effect of CD4 count on a positive TST, QFT or TSPOT test result. Prevalence odds ratio and 95% confidence intervals were calculated, median values were used to convert continuous variables to categorical variables in all instances. We defined a p-value of < 0.05 as being statistically significant.

RESULTS

Out of a cohort of 546 HIV-discordant couples currently enrolled at ZEHRP 403 couples met the prescreening criteria for study enrollment. The cross section for this study consists of 298 discordant couples or 596 study subjects **(Figure 1)**. The median age of study participants was 33 years (range 17-59 years), median monthly income was the equivalent of US $32 (range US $ 0-1908). Women were significantly more likely to be HIV positive than men (60% vs 40%, $p<0.0001$). Median CD4 count among HIV positive persons was 388 cells/ µl, (range 51-1330 cells/ µl). None of the study subjects were on antiretroviral therapy. Demographic and clinical data for study subjects is summarized in **Table 1.**

Diagnostic Test Results:

Tuberculin Skin Test (TST):

A total of 331 (55.5%) persons had a positive TST, 252 (42.3%) had a negative TST, and 13 (2.2 %) did not return to have their TST read. The median TST reading for persons with HIV was 18 mm and for persons without HIV was 16.5mm (p-value=0.05).

QuantiFERON-TB Gold in Tube (QFT) Test:

280 (47.0%) persons had a positive QFT, 281 (47.2%) had a negative QFT and 35 (5.8%) had an indeterminate test. Among persons with a positive QFT, persons without HIV secreted a higher level of Interferon γ 3.5 IU/ml in comparison to people who had HIV 1.8 IU/ml (p-value =0.0001).

TSPOT.TB (TSPOT) Test:

A total of 265 (44.5%) had a positive TSPOT, 313 (52.5%) had a negative test, and 18 (3.1%) had an indeterminate result. Among persons with a positive TSPOT, HIV positive individuals exhibited 44.2 spots/ml and HIV negative persons had 53 spots/ml (p-value=0.3). HIV positive persons were significantly less likely to have a positive TST, QFT or TSPOT result **(Table 2)**.

Indeterminate Test results with IGRAs:

All 18 indeterminate TSPOT results occurred due to technical errors resulting in a high value in the negative control well. Five of the 35 indeterminate test results with the QFT occurred because of inadequate interferon-γ release in response to the positive control. All five of these cases occurred among HIV positive individuals and the median CD4 count was 264 cells/µl.

Test results stratified by CD4 counts

Among HIV –seropositive individuals, subjects with a CD4 count <388 cells/ µl were less likely to have a positive test with TST and QFT, when compared to subjects with CD4 count $\geq$ 388 cells/ µl. This difference was not seen with TSPOT **(Figure 2)**.

Concordance Between Diagnostic Tests for Latent TB Infection:

Concordance between TST and QFT, TST and TSPOT and QFT and TSPOT was measured among HIV positive and HIV negative individuals. Overall concordance was moderate and there was no difference when comparing concordance between HIV positive and HIV negative subjects. **(Table 3)**. For the TSPOT test, concordance between

readings with a magnifying lens verses an ELISPOT reader was moderate κ=0.67 (95% CI 0.61-0.73).

Risk factors for a positive diagnostic test for LTBI:

Univariate analysis was performed and HIV status was found to be a risk factor for a positive test for TST(OR=2.87, 95% CI=1.94-3.97), QFT (OR=2.18 95 % CI=1.48-2.99) and TSPOT (OR=1.97 95% CI=1.26-2.53). In addition males were more likely to have a positive TST (OR=1.97 95% CI 1.39-2.79) and TSPOT (OR=1.80 95% CI 1.27-2.55), however this effect was only seen on univariate analysis. BCG vaccination, household contact with a case of active TB, history of prison stay and income were not found to be risk factors for a positive test with TST, QFT or TSPOT. **(Table 4)**

On multivariate analysis subjects who were HIV negative were more likely to have a positive TST, QFT and TSPOT, when controlling for age and gender. **(Table 5,6,7)**
We then performed multivariate analysis on HIV positive individuals alone and found that subjects with a CD4 count $\geq$388 cells/µl were more likely to have a positive TST (OR 1.76 95% CI 1.10-2.82) or QFT (OR 1.71, 95% CI 1.06-2.77). This association was not seen with TSPOT (OR1.20, 95% CI 0.74-1.94). **(Table 8,9,10)**

DISCUSSION

This is the first study to investigate all three diagnostic tests for latent tuberculosis infection (TST, QFT and TSPOT) in an HIV positive population and to compare results to an HIV negative partner as a control group. Using HIV discordant couples allowed us to compare study subjects with similar demographic, socioeconomic and exposure data to determine what the effect of HIV is on LTBI test positivity. The TST has been shown to have decreased sensitivity among HIV positive persons but there have been limited data on the sensitivity of IGRAs in this population [29-31]. Since one of the strategies to control tuberculosis is diagnosis and treatment of LTBI particularly among HIV-infected persons, we sought to assess IGRAs for the diagnosis LTBI among HIV-infected persons.

The prevalence of a positive test with TST, QFT and TSPOT was significantly lower among HIV positive persons, when compared to HIV negative persons, suggesting these tests do not perform as well in HIV positive persons. Induration with TST was greater in HIV negative than in HIV positive persons and amount of interferon-gamma release with QFT was greater among HIV negative persons than HIV positive persons. There was no difference in spot forming cells for TSPOT between HIV positive and HIV negative persons.

On multivariate analysis being HIV negative was the only predictor for a positive test result, with TST, QFT and TSPOT, while controlling for other risk factors. A study from Africa compared QFT in HIV positive and negative subjects within the same population

and was unable to show a difference in rates of test positivity based on HIV status. However that study had a small sample size and the HIV negative (controls) were not as closely matched as in our study [18]. A study from Spain did show that rates of positive QFT were lower among HIV positive than HIV negative individuals but TSPOT was not evaluated [32].

We performed multivariate analysis to assess whether CD4 count was an independent predictor of TST, QFT or TSPOT positivity. Among HIV-seropositive persons, a CD4 count $\geq$388 cells/µl was associated with a positive TST and QFT. CD4 count was not a predictor for a positive test result with TSPOT. This finding suggests that TSPOT may work better than TST or QFT among HIV positive individuals as results are not dependant on a patient's CD4 count. A study from Uganda also found that the rate of positive TSPOT results were comparable in HIV positive patients with CD4 counts < 100, 100–250 and >250 cells/µl, however no multivariate analysis was performed in this study [33].

We compared concordance between TST and IGRAs, and between QFT and TSPOT and found fair concordance. There was no difference in concordance when we looked at just HIV-seropositive persons, or only HIV negative persons. Several other studies have shown fair to poor concordance in HIV [19-21]. Since concordance depends on prevalence, values from developed countries are lower than those from high incidence developing countries and are difficult to compare [14-18].

Most studies in HIV positive individuals have shown a large number of indeterminate results and these have been associated with low CD4 counts [16,17]. Our study had a low incidence of indeterminate test results and most of these occurred due to a high interferon reading in the negative control well, a finding that occurs due to technical problems. Five cases of indeterminate QFT results occurred due to inadequate interferon in the positive control. All of these occurred in HIV positive individuals. We had fewer indeterminate test results with TSPOT than with QFT.

Our study has several limitations. The study was conducted in a developing country with a high incidence of TB and HIV and therefore may not be generalizable to the developed world. The median CD4 count in our population was high (388 cells/μl) and no study subject was on HAART. It is difficult to assess how these tests would perform at lower CD4 counts. Our study used the HIV negative partner as a control, to estimate the baseline prevalence of latent tuberculosis in the study population. We realize that both partners within a couple would not have identical exposures to tuberculosis, and that this may be considered a shortcoming of our study. However we do feel that their exposures would be similar and therefore both groups should have a similar rate of LTBI. Future studies could ask for more detailed exposure histories. Our study had a cross sectional design and therefore we don't have longitudinal data to determine whether a positive IGRA predicts future risk of active TB. However longitudinal studies are not practical due to the need for a large sample size and follow up over several years.

The advantage of conducting a study in a high incidence country is that we have a large number of positive IGRA test results and therefore have more power for analysis of risk factors. In addition, we used a unique study design where we matched each HIV positive person to their HIV negative domestic partner and then carried out a matched analysis using unconditional logistic regression. This is the first study to show that TSPOT may have an advantage over TST and QFT for diagnosis of LTBI among HIV positive persons.

In conclusion, we found that HIV positive status does decrease the sensitivity of TST, QFT and TSPOT. On multivariate analysis HIV status was the only predictor of test positivity for TST, QFT and TSPOT. Among HIV positive subjects a CD4 count >388 cells/ μl was associated with a positive TST and QFT, but not TSPOT. Based on the findings of our study it appears that TSPOT may be a better diagnostic test for LTBI in HIV positive persons as results do not depend on CD4 count. Further research to evaluate use of TSPOT at lower CD4 counts and in other immunocompromised states is needed.

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Table 1: Clinical characteristics of HIV positive and HIV negative subjects enrolled in the study (n=596)

Characteristics of study subjects	**HIV positive n=298**	**HIV negative n=298**	***p-value***
Age median and range	32 (18-59 years)	34 (17-59 years)	0.045
Male Gender	118 (40%)	180 (60%)	<0.0001
History of prior incarceration	16 (5%)	18 (6%)	0.7
History of BCG vaccination	223(75%)	220 (74%)	0.9
History of household TB exposure in past 30 days	33 (11%)	37(12%)	0.8
Median monthy income $	32	32	0.6
Median CD4 count	388 cells/µl		

BCG: Bacillus Calmette-Guérin
HIV: Human Immunodeficiency Virus

Table 2: Prevalence of a positive diagnostic test for Latent Tuberculosis Infection (LTBI) stratified by HIV status (n=596)

Diagnostic Test for LTBI	HIV positive n=298	HIV negative n=298	p-value
TST	128 (43%)	203 (69%)	0.0003
QFT	109 (37%)	171 (58%)	0.006
TSPOT	109 (37%)	156 (53%)	0.005

TST: Tuberculin Skin Test
QFT: QuantiFERON-TB Gold In Tube Test
TSPOT: TSPOT.TB Test
HIV: Human Immunodeficiency Virus

Table 3: Concordance between the three diagnostic tests described in all subjects, HIV seropositive subjects and HIV seronegative subjects

Test in HIV positive subjects (n=298)	**Agreement (%)**	**κ**	**95% Confidence Interval**
TST vs QFT	75%	0.53	0.43, 0.64
TST vs TSPOT	76%	0.4	0.3, 0.52
TSPOT vs QFT	74%	0.37	0.26, 0.49
Test in HIV negative subjects (n=298)	**Agreement (%)**	**κ**	**95% Confidence Interval**
TST vs QFT	72%	0.44	0.33, 0.55
TST vs TSPOT	70%	0.3	0.2, 0.4
TSPOT vs QFT	75%	0.46	0.35, 0.57

TST: Tuberculin Skin Test
QFT: Quantiferon-TB Gold In Tube Test
TSPOT: TSPOT.TB Test
HIV: Human Immunodeficiency Virus

Table 4: Univariate Analysis to evaluate risk factors for a Positive Diagnostic Test for LTBI, using TST, QFT and TSPOT.

Risk factor	**TST OR (95% CI)**	**QFT OR (95% CI)**	**TSPOT OR (95% CI)**
Age ≥32 vears	0.80 (0.56, 1.13)	0.91 (0.65, 1.29)	1.09 (0.77, 1.54)
Male Gender	**1.97 (1.39, 2.79)**	1.36 (0.97, 1.93)	**1.80 (1.27, 2.55)**
BCG vaccination	0.92 (0.57, 1.50)	1.09 (0.68, 1.76)	0.72 (0.45, 1.16)
House hold contact	1.45 (0.84, 2.48)	0.68 (0.40, 1.16)	1.48 (0.86, 2.56)
Prison	1.66 (0.76, 3.59)	1.29 (0.62, 2.69)	0.88 (0.42, 1.83)
Income <$35	0.69 (0.46,1.00)	0.88 (0.62, 1.25)	0.82 (0.57, 1.17)
HIV negative	**2.87 (1.94, 3.97)**	**2.18 (1.48, 2.99)**	**1.97 (1.26, 2.53)**

Table 5: Multivariate Analysis, using Conditional Logistic Regression to evaluate Risk Factors for a Positive Diagnostic Test for Latent Tuberculosis Infection using TST

Risk factors for positive TST	**Odds Ratio**	**95% Confidence Interval**
Age <32 years	0.98	0.36-2.62
Male Gender	1.33	0.79-2.22
HIV negative*		
Age <32 years	**1.86**	**1.06-2.67**
Age ≥ 32 years	**3.67**	**1.60-8.39**

*Interaction between age and HIV
TST: Tuberculin Skin Test

Table 6: Multivariate Analysis, using Conditional Logistic Regression to evaluate Risk Factors for a Positive Diagnostic Test for Latent Tuberculosis Infection, using QFT

Risk factors for positive QFT	Odds Ratio	95% Confidence Interval
Age <32years	0.56	0.24-1.36
Male Gender	0.92	0.55-1.53
HIV negative	**2.22**	**1.42-3.46**

QFT: Quantiferon-TB Gold In Tube Test
HIV: Human Immunodeficiency Virus

Table 7: Multivariate Analysis, using Conditional Logistic Regression to evaluate Risk Factors for a Positive Diagnostic Test for Latent Tuberculosis Infection, using TSPOT

Risk factors for positive TSPOT	Odds Ratio	95% Confidence Interval
Age <32 years	0.98	0.42-2.28
Male Gender	1.59	0.92-2.76
HIV negative	**1.79**	**1.16-2.77**

TSPOT: TSPOT.TB Test
HIV: Human Immunodeficiency Virus

Table 8: Multivariate Analysis, using Unconditional Logistic Regression to evaluate Risk Factors for a Positive Diagnostic Test for Latent Tuberculosis Infection, using TST among HIV positive individuals

Risk factors for positive TST	Odds Ratio	95% Confidence Interval
Age <32 years	1.44	0.88-2.36
Male Gender	1.38	0.83-2.36
CD4 count ≥388cells/ µl	**1.76**	**1.10-2.82**

Table 9: Multivariate Analysis, using Unconditional Logistic Regression to evaluate Risk Factors for a Positive Diagnostic Test for Latent Tuberculosis Infection, using QFT among HIV positive individuals. (n=298)

Risk factors for positive QFT	Odds Ratio	95% Confidence Interval
Age <32 years	1.16	0.70-1.93
Male Gender	1.23	0.74-1.93
CD4 count ≥388cells/ μl	**1.71**	**1.06-2.77**

Table 10: Multivariate Analysis, using Unconditional Logistic Regression to evaluate Risk Factors for a Positive Diagnostic Test for Latent Tuberculosis Infection, using TSPOT among HIV positive individuals. (n=298)

Risk factors for positive TSPOT	Odds Ratio	95% Confidence Interval
Age <32 years	1.36	0.83-2.25
Male Gender	1.47	0.88-2.45
CD4 count ≥388cells/ μl	1.20	0.74-1.94

Figure 1: Enrollment of Study Subjects from ZEHRP Cohort

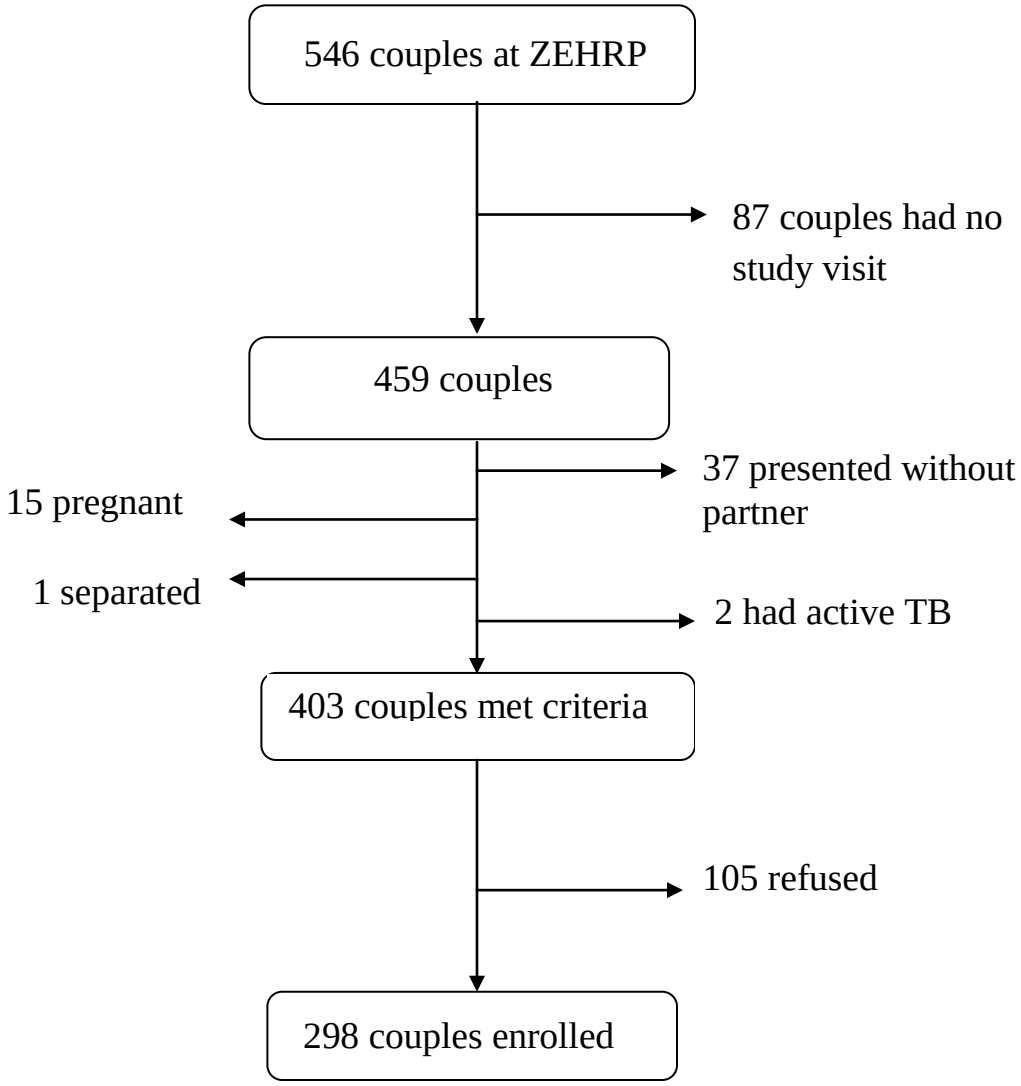
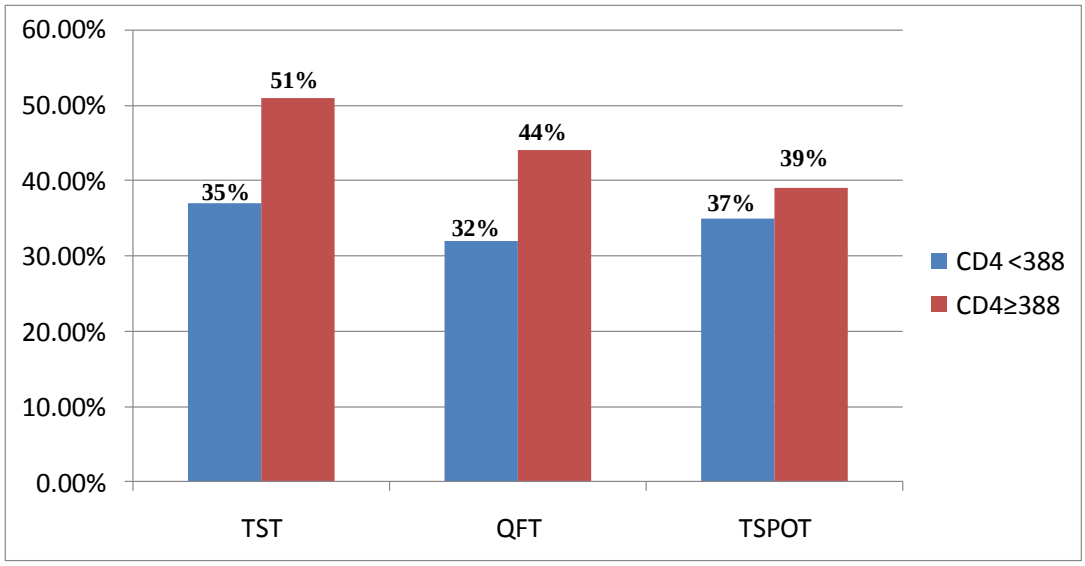


Figure 2: Positive diagnostic test for latent tuberculosis infection stratified by CD4 count (n=298)



p-value =0.02 p-value=0.02 p-value=0.46

APPENDIX

A. Sample size calculation using the precision method (Half width of the 95% confidence interval)

Sample Size	**Half width of 95% CI** **(Prevalence of positive TST in HIV positive 30%)**	**Half width of 95% CI** **(Prevalence of positive TST in HIV negative 60%)**
100	9	10
200	6.5	7
300	**5.3**	**5.7**
400	4.5	4.8
500	4.0	4.2
600	3.7	4