SCREENING FOR PULMONARY TUBERCULOSIS AMONG HIV-INFECTED PREGNANT WOMEN IN WESTERN KENYA

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DECLARATION

This dissertation is my original work and has not been presented elsewhere. References to work done by others have been clearly indicated.

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CERTIFICATE OF AUTHENTICITY

This is to certify that this dissertation is the original work of Dr Rose Jepchumba Kosgei Master of Medicine student in Department of obstetrics and Gynaecology, Registration number H58/70755/2007 University of Nairobi (2007-2011). The research was carried out in the department of obstetrics and Gynaecology, School of Medicine, College of Health Sciences. It has not been presented in any other university for award of a degree.

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DEDICATION

To my beloved Mum, Hellen Jeruto Kosgei and Dad Charles Barmao Kosgei Salil

LIST OF ABBREVIATIONS

AAFB- Acid-Alcohol-Fast Bacillus

AIDS- Acquired Immune Deficiency Syndrome

AMPATH- Academic Model Providing Access To healthcare

ARV- Antiretroviral

ATS- American Thoracic Society

CDC-Centres for Disease Control and Prevention

CXR- Chest Radiograph

DLTLD- Division for Leprosy Tuberculosis and Lung Disease

HIV- Human Immunodeficiency Virus

IPT- Isoniazid Preventive Therapy

IREC- Institutional Research and Ethics Committee

IRIS- Immune Reconstitution Inflammatory Syndrome

LTBI- Latent Tuberculosis Infection

MGIT- Mycobacteria Growth Indicator Tube

MTRH- Moi Teaching and Referral Hospital

PA- Posterior Anterior

PI- Principal Investigator

PMTCT- Prevention of Mother-To-Child Transmission of HIV/AIDS

PPD- Purified Protein Derivative

PTB- Pulmonary Tuberculosis

TB- Tuberculosis

TST-Tuberculin Skin Test

TT- Tetanus Toxoid

USAID- United States Agency for International Development

WHO- World Health Organization

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ABSTRACT

Background: Pulmonary tuberculosis (PTB) is the most common opportunistic infection affecting HIV-infected patients including pregnant women, yet screening for PTB is not routinely or systematically done in most Prevention of Mother-To-Child Transmission of HIV/AIDS (PMTCT) programs. Diagnosis of PTB among HIV-infected pregnant women poses unique challenges including: confusion of constitutional symptoms of tuberculosis (TB) with those of early pregnancy, reluctance to perform radiographic screening, lack of access to TB culture in resource-poor settings and the paucibacillary nature of PTB among HIV-infected patients leading to lower smear positivity rates than their negative counterparts.

Objectives: Among HIV-infected pregnant women participating in a standard PTB screening program in Western Kenya to determine the: 1) prevalence of latent tuberculosis infection (LTBI); 2) prevalence of active PTB and 3) sensitivity, specificity and predictive value of the diagnostic tests in the diagnosis of active PTB.

Methods: This is a cross-sectional study with an analytic component carried out in Eldoret and Busia PMTCT clinics within the USAID-AMPATH partnership; Western Kenya. All eligible HIV-infected pregnant women receiving care during the five month study period and consented underwent: a standardized structured symptom and sign screen, tuberculin skin test (mantoux), single view posterior anterior dose regulated chest radiograph (CXR) performed after the first trimester and sputum smear and cultures when cough was present.

Results: A total of 190 patients who met the eligibility criteria and consented were enrolled. The study participants had a mean age of 27 years and a median (range): age of 25(15-43) years; parity of 2 (0, 10); gravidity of 3 (1, 11); gestation at enrolment of 26(10, 41) weeks and CD4 counts of 440 (24, 1408) cells/µl. Only 20% of the study respondents had a positive symptom and sign screen. The prevalence of: LTBI as defined by a positive mantoux skin test was 40% and of presumptive active TB as defined by suggestive chest radiograph findings was 5%. There was a notable representation of milliary TB; of the 10 cases with suggestive radiological features of active TB 3 had a milliary picture. None of the 28 samples of sputum's collected were positive for Ziehl-Neelsen staining or for mycobacterium tuberculosis culture. The role of different screening procedures was inconclusive due to a small number of those with active TB and the fact that there was no microbiological evidence of active TB.

Conclusion and Recommendation: LTBI infection is common in this population with a remarkable percentage of chest radiographs suggestive of TB and a notable milliary presentation. TB screening should be routine in all PMTCT care programs in high TB and HIV burden areas. More studies are required to determine best screening protocols as well as feasibility of Isoniazid Preventive Therapy (IPT) in this population.

CHAPTER 1: BACKGROUND AND LITERATURE REVIEW

1.1 Background and Literature review

Mycobacterium Tuberculosis, the bacterium that causes tuberculosis (TB) is estimated to infect one-third of the world's population[1], with 95% of TB cases and 98% of TB deaths occurring in resource limited countries. TB is the leading cause of death in patients living with HIV/AIDS as well as the leading cause of death in women of reproductive age[2]. Kenya is ranked 13th among the world's 22 countries with high TB burden[3]. In 2007, there were 116,723 cases of TB reported in Kenya; 79% of whom were HIV-infected[4]. TB and HIV are intimately linked infections; posing a significant threat to the gains made in scaling up prevention, care and treatment programs for people living with HIV/AIDS[5] . Pregnancy alone has not been shown to influence the pathogenesis of TB or the likelihood of progression from latent to active disease[6].

The development of TB disease has been linked to increased HIV replication and reduced CD4 cell counts, contributing to the progression of HIV infection[7]. On the other hand, the immunodeficiency induced by HIV leads to increased rates of TB infection if exposed and increased rates of TB disease once infected. HIV is associated with a 20-fold increased risk of reactivation of latent TB infection (LTBI) [8-9]. HIV infection and TB during pregnancy are a particularly deadly combination and are independent risk factors for maternal mortality[10-12]. A challenge facing PMTCT care programs is how to make a diagnosis of active TB

both due to limitations in the presently available diagnostic tests as well as due to lack of access to many of these diagnostic tests.

TB is the most frequently encountered HIV related opportunistic infection during pregnancy and the commonest cause of maternal mortality in this group of patients[13-18]. Most maternal deaths caused by TB, are attributable to co-infection with HIV and depending on the setting, ranges from 14%-54% [12, 19-20]. Active TB in pregnant women has serious consequences which include: mother-to-child TB transmission–either intrauterine or postpartum, up to 3.4-fold increased risk of maternal death[20-22], preterm delivery and intrauterine growth retardation [23]. In addition, is the risk of immune reconstitution inflammatory syndrome (IRIS), among HIV-infected pregnant women with undiagnosed active TB initiated on highly active antiretroviral therapy (HAART); for prevention of mother-to-child transmission (PMTCT) of HIV virus. IRIS is described as a collection of inflammatory disorders associated with paradoxical worsening of pre-existing infectious processes following the initiation of HAART among HIV-infected patients [24-31].

The rate of TB disease among HIV-infected pregnant women is 10 times higher than that among their HIV-uninfected counterparts [32-33]. In Kenya there are no studies reporting the prevalence of active TB in pregnancy. The Kenya Ministry of Health, Division of Reproductive Health estimates that at least one out of eight HIV-infected pregnant women could be co-infected with TB[34]. The incidence of

active TB among HIV-infected pregnant women varies in different settings and cohorts. In a Kenyan cohort, the incidence of TB was 11 per 100 person-years among HIV-infected pregnant women[35]. In another study performed in Durban, South Africa, TB occurred in 0.1% and 0.6% of pregnant women in 1996 and 1998 respectively with overall TB rates for HIV-uninfected pregnant women of $72.9/10^5$, and for HIV-infected pregnant women of $774.5/10^5$ [11].

The presentation of TB among pregnant women is similar to that among non-pregnant women[36] but diagnosis may be delayed by the non-specific nature of early symptoms[37] such as frequency of malaise and fatigue in pregnancy which mimic early symptoms of active TB disease[38]. Pulmonary TB (PTB) is the most common site of presentation in pregnancy and is the form that is transmittable [39]. Its diagnosis is complicated by several factors including: 1) Chest radiographs are usually prescribed cautiously in pregnancy, due to the concern regarding teratogenicity during the first trimester[37], 2) culture facilities to diagnose smear negative culture positive disease are not readily available in the developing world and 3) sputum smear microscopy is often not requested. Other studies, demonstrate less significant symptoms in pregnant women with TB. Therefore, if routine screening is not performed antenatally or postnatally most pregnant women will not have their TB diagnosed early for prompt treatment hence increasing the risk of TB transmission to the: foetus, newborn, obstetric ward, family and community at large[40] .

One-third of the world's population is infected with *M. tuberculosis*[1]. In most cases with an intact immune system, the infected individual mounts an effective immune response that culminates in granuloma formation around the infective foci and subsequent arrest of disease progression. Bacilli within these granulomas are not killed but, instead, remain dormant this is termed a LTBI [41-42]. Approximately 10% of latent infections reactivate, resulting in active TB disease months to years after the initial infection[42]. The risk of reactivation increases from 5 to 15% annually in persons co-infected with human immunodeficiency virus[43]. Routine testing for LTBI in pregnant women is not indicated in HIV-uninfected pregnant women. However screening for LTBI is recommended in pregnancy in patients with immunosuppressive conditions such as HIV infection. This is because of the associated increased risk for progression of LTBI to active TB disease. A decision to screen LTBI assumes that a decision to treat promptly with Isoniazid Preventive Therapy (IPT) will follow a positive screen[44]. IPT has been shown to reduce reactivation of LTBI to active TB disease. Widespread use of IPT has been limited due to challenges of ruling out active TB disease in pregnancy. In settings where active disease can be ruled out, IPT is recommended to patients at high risk of progressive disease developing such as HIV-infected pregnant women[45] .

PTB screening in the antenatal clinic setting is an acceptable intervention as illustrated in Kasungu District, Malawi[46] and it has been recently incorporated into Kenya's focused antenatal care guidelines[34]. In high TB and HIV burden areas,

screening for TB should be part of PMTCT programs as demonstrated successfully in an antenatal clinic in Johannesburg, South Africa, where pregnant women found to be HIV infected, during a routine antenatal care voluntary testing program were routinely screened for symptoms of TB[18]. Despite this evidence most PMTCT programs have not integrated active systematic screening of TB into care. This is largely because screening and diagnosis of TB in pregnancy still remains a big challenge. The screening and diagnostic approach for the evaluation of PTB is however unchanged by pregnancy. Mantoux test is used for screening LTBI. Diagnostic evaluations for suspected active TB include: Acid-Alcohol-Fast Bacillus (AAFB) stain and culture of clinical material in combination with chest radiography with appropriate shielding when indicated in pregnancy[47]. The diagnostic challenges include the limitation of the presently available diagnostic tests as well as the need for training of healthcare providers as to the safety of these methods and the importance of screening in pregnancy.

The Mantoux test also known as tuberculin skin test (TST) or purified protein derivative of tuberculin (PPD) is used to screen for LTBI by identifying individuals with previous sensitization to mycobacterial antigens. The role of PPD differs according to setting; it has been shown to be of limited value in screening for PTB in populations with either a high prevalence of TB or who have received BCG immunization at an early age [48] while on the other hand its useful as a screening tool in low TB burden areas [40]. There are no reported harmful effects to the mother or foetus with PPD test [49.] Physiologically PPD induces a type IV

hypersensitivity reaction due to mononuclear cellular infiltration and inflammation. This reaction peaks at 48-72 hours, manifested as a skin induration. The intensity of this local reaction depends on the person's previous exposure to mycobacterium TB. For patients who are HIV-infected an induration greater than or equal to 5mm is considered a positive PPD. When a skin test is interpreted as positive, the patient should receive a chest radiograph[50] and physical examination to rule out active PTB infection.

Responsiveness to the PPD skin test is dependent on normal T-cell number and function. Decreased T-cell function may result in the inability to mount an immune response, resulting in anergy. Anergy is defined as the absence of the capacity to express delayed-type hypersensitivity skin test reactivity to normally encountered antigens[51]. Patients with AIDS or other immunologic disorders may not react due to their immune dysfunction. Often, to assure an active immune system, a control is placed at the same time as the PPD. Control skin tests, such as mumps, tetanus or Candida, use antigens that most people have been exposed and will form a reaction to when given an intradermal injection. A reaction at the site of the control skin test suggests an active, intact immune system, and is used to determine that a negative PPD is truly indicative of no TB infection.

There is controversy in the usefulness and effectiveness of anergy testing to assure an active immune system. Earlier studies indicated that, anergy panels

are useful in the interpretation of a negative PPD[50, 52.] The Centers for Disease Control and Prevention Advisory Council for the Elimination of Tuberculosis revised its guidelines on TB screening in high risk populations, stating that 'the scientific basis for anergy testing is weak and is generally not part of screening for TB infection. However, those at high risk for TB may be evaluated for anergy, taking into account that anergy practices are not well standardized'[52] . In pregnancy the combination of a negative symptom screen and a negative PPD occurs in 25% of persons with active TB[40].

Sputum microscopy is the present examination to diagnose contagious forms of PTB but may only identify 50-60% of cases of active PTB in well-equipped laboratories[53]. In a study to determine the diagnostic accuracy of sputum microscopy for active case finding of HIV-associated PTB using TB culture as the reference standard, the overall sensitivity of sputum microscopy was 61.8% and specificity 99.7%. In this same study sputum microscopy sensitivity varied from 22.6% to 94.2%, positive and negative predictive values were 84.5% and 99.1%, respectively[54]. Poor access to high-quality microscopy services contributes to even lower rates of AAFB detection in low-income countries. Furthermore, in countries with high prevalence of both PTB and HIV infection, the detection rate is even lower owing to the paucibacillary (having few bacilli) nature of PTB in patients with HIV infection[55].

Sputum culture, though the gold standard for diagnosis, is frequently unavailable in resource poor settings[53]. Culture in cases of active disease is found to be 81% sensitive and 98.5% specific[56]. With newer culture systems like the Mycobacteria Growth Indicator Tube (MGIT), growth can be detected in a mean time of 15.3 days which is lower than the commonly used Lowenstein-Jensen medium which has a mean time of 25.4 days. Although MGIT technology can decrease culture turnaround time, the limitations of culture recovery in terms of specimen collection and recovery of paucibacillary samples of organisms is still a problem[57].

Lastly, there is reluctance to utilize chest radiographs in pregnancy because of fears of associated teratogenicity in early pregnancy[37]. In addition, chest radiography does not supply a specific microbiologic diagnosis but rather identifies patients for whom more aggressive screening in terms of specimen collection is needed. The overall sensitivity and specificity for chest radiographs in diagnosing PTB has been documented in some studies to be between 51 and 78% [58]. With appropriate shielding, the benefit derived from an appropriately indicated chest radiograph out weights the risk of foetal damage from low-dose radiation[50] and one single view posterior anterior shielded chest radiograph is safe to use after the first trimester[59].

1.2 Justification

Despite TB being the most common opportunistic infection affecting HIV/AIDS patients, screening and diagnosis for TB is rarely routinely or systematically performed for HIV-infected pregnant women in most PMTCT programs. This is despite the fact that both TB and HIV independently increase maternal and perinatal morbidity and mortality. Thus, among PMTCT clinics where HIV care is routinely performed this represent a perfect opportunity to screen for this common and potentially devastating opportunistic infection. Diagnosis of active PTB among HIV-infected pregnant women is essential because pulmonary system is the commonest site of disease and is the form that is transmissible to the newborn. Diagnosis of PTB however poses unique challenges; symptoms of PTB are non-specific and mimic the constitutional symptoms that are often ascribed to the pregnancy itself. Diagnostic tests for TB infection (the PPD) are not widely available though safe for mother and foetus and are of limited value in high TB burden areas. Smear diagnostics present no risk to the mother but may miss earlier cases and are associated with low detection rate owing to the paucibacillary nature of PTB in patients with HIV infection. Culture is rarely available due to cost. Finally there exists a general reluctance in obtaining chest radiographs due to perceived risk to the foetus.

There are no studies reporting on the prevalence of active TB among HIV-infected pregnant women in Kenya. Screening of LTBI among HIV-infected

pregnant women is not done routinely and there are no national guidelines on Isoniazid Preventive Therapy (IPT) within PMTCT programs. This clinic based cross-sectional study was designed to identify a feasible screening approach and to describe the prevalence of LTBI and active PTB among HIV-infected pregnant women being cared for in the United States Agency for International Development - Academic Model Providing Access To Healthcare (USAID-AMPATH) Partnership referred to as AMPATH hereafter PMTCT program in Western Kenya. Ascertaining the TB status in this group of patients is for the general health of the mother and baby. Missed TB disease has the risk of maternal or foetal death as well as foetal growth retardation or perinatal TB. In addition, undiagnosed TB may in the face of prescribed antiretroviral therapy result in a condition known as Immune Reconstitution Inflammatory Syndrome (IRIS). In the AMPATH program IPT has been demonstrated to reduce the incidence of TB in HIV infected patients but has to date never been offered to pregnant women in the program. Information as to the prevalence of both TB infection as well as TB disease in pregnant HIV-infected women is urgently needed in order to design appropriate screening paradigms and preventive therapy enrolment programs for reduction of TB disease. In addition this study will provide information necessary to design an effective TB prophylaxis (IPT) program that can be integrated into PMTCT programs. IPT reduces the risk of progression to active TB in those individuals with latent TB but requires ruling out of active TB disease. Due to paucity of studies on this subject, information gained from this study will provide pilot data to develop other studies and assist in the

development and strengthening of regional (AMPATH) and national (Kenya) screening guidelines for HIV-infected pregnant women in high TB and HIV burden areas. The information may also be used to inform screening guidelines for other high TB and HIV burden settings as well.

1.3 Research questions

1. What is the prevalence of latent TB infection (LTBI) as defined by Mantoux Skin Test (PPD) among HIV-infected pregnant women in Western Kenya?
2. What is the prevalence of active pulmonary tuberculosis (PTB); as defined by sputum microscopy, sputum culture and chest radiographs among HIV-infected pregnant women in western Kenya?

1.4 Broad Objective

To determine the prevalence of LTBI and active PTB among HIV-infected pregnant women being cared for in Busia and Eldoret AMPATH clinics, Western Kenya using clinical, laboratory and radiological assessments.

1.5 Specific Objectives

1.5.1 Primary Specific Objectives

1. To determine the prevalence of LTBI among HIV-infected pregnant women, participating in a standard screening program for PTB in Western Kenya
2. To determine the prevalence of active PTB among HIV-infected pregnant women participating in a standard screening program for PTB in Western Kenya

1.5.2 Secondary Specific Objective

1. To determine the sensitivity, specificity and predictive value of the diagnostic tests in the diagnosis of active PTB among HIV-infected pregnant women participating in a standard screening program for PTB in Western Kenya

CHAPTER 2: METHODS

2.1 Study Site and Setting

This study was carried out in western Kenya in two of the forty six sites of the AMPATH program. AMPATH is a primary care program that offers comprehensive HIV care in Western Kenya [60-63]. The two sites were Eldoret and Busia PMTCT clinics. The Eldoret clinic is housed by Moi Teaching and Referral Hospital (MTRH) which is the teaching hospital for The Moi University School of Medicine. It has three modules offering adult HIV care. As at November 2008 there were 15,000 HIV-infected adult patients; 10,000 female and 5,000 male enrolled in the Eldoret clinic. The Busia clinic is housed by the Busia District Hospital situated at the Kenya Uganda border, and is located west of the Eldoret clinic about 200 kilometres away. As of the end of November 2008 there were 6,000 HIV-infected adult patients; 4,000 female and 2,000 male enrolled in the Busia clinic. The two sites were chosen for the study for the following reasons; 1) these two clinics represent the busiest PMTCT programs in within the AMPATH care program (average of 30 patients per month each), 2) both clinics have functional TB smear microscopy laboratory and 3) both sites have functional radiography suites with capability of chest radiograph machines, with appropriate shielding for pregnant women.

Routinely in the program, pregnant women who test HIV positive in the antenatal clinic are referred to AMPATH PMTCT clinic, for management in accordance to the set protocols. Recruitment of patients for this study was done in PMTCT

clinic. As standard of care on enrolment, an initial encounter form is completed which has a section on respiratory symptom screen. Pregnant women, who are clinically suspected to have TB, are requested to produce sputum for microscopy and those who are smear positive are commenced on anti-TB treatment as per protocol. On the other hand, those whose sputum's are all smear negative are cultured and treatment instituted appropriately based on clinical impression. Dose regulated shielded chest radiographs are performed as deemed appropriate by the clinician. Chest radiographs are interpreted on-site by clinicians (physicians and clinical officers) in the PMTCT clinic; abnormal radiographs that do not portray a typical pattern are sent for interpretation by radiologists (for the purposes of this study, chest radiographs were interpreted by clinicians on site for patient care but also reviewed by a radiologists who was blinded to the clinician's interpretation for the study purposes).

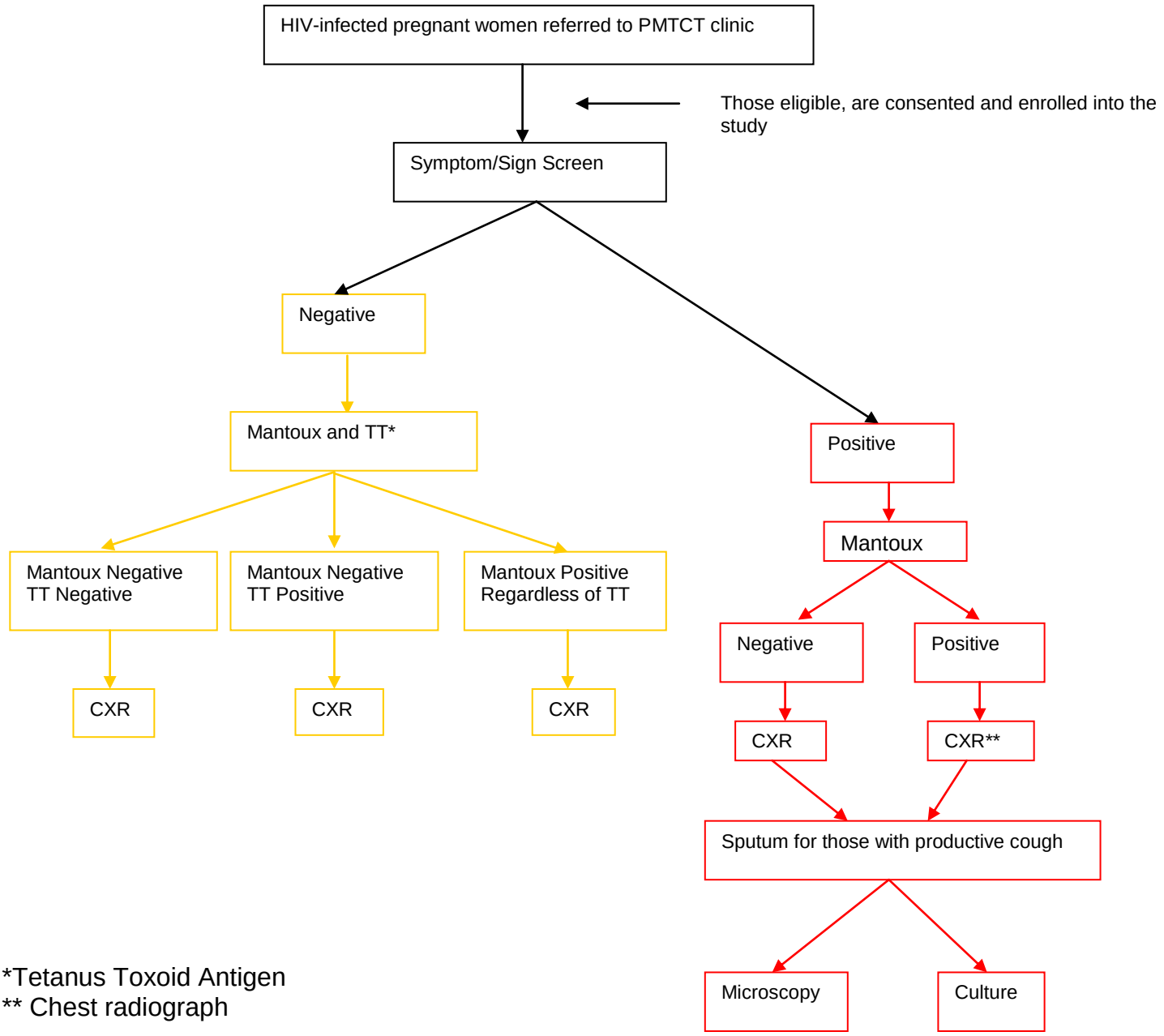
The protocol for sputum collection in the PMTCT clinic follows the guidelines of the Division of Leprosy Tuberculosis and Lung Disease (DLTLD) national program: initial spot sample is taken on the first contact with the patient, a morning specimen is brought by the patient the next day and a second spot specimen is collected. All smear negative specimens receive a free sputum culture for TB. Time to positive culture average 8 days on a Mycobacteria Growth Indicator Tube (MGIT) system and a backup system of Lowenstein-Jensen media is also available; specimens are not considered culture negative until negative in both specimens (42 days).

2.2 Study Design

This is a clinic-based cross-sectional study with an analytic component. Written consent was obtained from eligible patients and enrolled into the study in the PMTCT clinic. A pre-tested standardized structured questionnaire and symptom/sign screen (appendix II) was completed. The following algorithm, figure 1 was followed depending on the outcome of the symptom/sign screen: 1) patients who had a positive symptoms/sign screen were administered mantoux Skin Test (PPD), underwent a single view posterior anterior (PA), dose regulated chest radiograph and were asked to produce sputum for microscopy and culture if they had a productive cough and 2) patients who had a negative symptom/sign screen were administered PPD and Tetanus Toxoid (TT) antigens for anergy panel and underwent a single view PA, dose regulated chest radiograph.

The primary outcome measures were the prevalence of LTBI and active PTB among HIV-infected pregnant women participating in a standard screening program for PTB. On the other hand the secondary outcome measure was the sensitivity, specificity and predictive value of the diagnostic tests used during the screening.

Figure 1: *Testing Algorithm*



*Tetanus Toxoid Antigen
** Chest radiograph

Patients enrolled to the study and diagnosed with active PTB were treated as per program protocol in the PMTCT clinic

2.3 Study Population

The study participants were; HIV-infected pregnant women being cared for in the Eldoret and Busia PMTCT AMPATH clinics, Western Kenya.

Inclusion criteria

1) In reproductive age, 15-49 years
2) HIV-infected pregnant women who were newly enrolled to the PMTCT program
3) ARV naïve at the time of enrolment
4) Able to provide written informed consent

Exclusion criteria

1) Patients who were on TB treatment at the time of enrolment
2) Patients who had a past history of Isoniazid Preventive Therapy (IPT)
3) Patients who were unable to return to the clinic after 48 hours for the reading of PPD and TT test skin antigens and collection of 2nd and 3rd sputum sample
4) Patients who presented in labour

2.4 Sample size calculation and sampling procedure

2.4.1 Sample size calculation

In calculation of the sample size an incidence of TB amongst HIV-infected women was considered as the proportion P, (12.5 %) based on the estimations by the Kenya ministry of Health Division of reproductive Health[34]. Using the formula below a sample size of 166 and 10 % of this was added to guard against

lost to follow up or incomplete data. A total sample size of 183 was obtained as shown below.

$$n = \frac{N \times Z^2 \times P(1-P)}{d^2 \times (N-1) + Z^2 \times P(1-P)}$$

N=Total Number of HIV Positive Women

d=absolute precision

p=expected proportion of in the population

Design effect = 1 (no change in variance)

$Z_{(1-\alpha)}$ =1.96

$$n = \frac{14{,}000 \times 1.96^2 \times 0.125 \times (1-0.125)}{0.05^2 \times (14{,}000-1) + 1.96^2 \times 0.125 \times (1-0.125)}$$

$$n = \frac{14{,}000 \times 0.420175}{0.05^2 \times 13{,}999 \times 0.420175}$$

$$n = \frac{5{,}882.45}{35.41768}$$

n=166

n=10% x 166+166

n=17+166

n=183

2.4.2 Sampling procedure

Consecutive sampling was used in this study; all the first 183 patients who met the eligibility criteria and gave consent formed the study sample. This sampling method was appropriate for this study because of the small numbers of patients

who were eligible (on average 30 HIV-infected pregnant patients get enrolled per month in each clinic) and it minimized selection bias by consecutively selecting every patient who met the eligibility criteria.

2.5 Data Collection and Management

2.5.1 Data Collection

Data for this study was collected prospectively between October 1st 2009 and 28th February 2010. Research assistants were two clinical officers (mid-level practitioners) and one nurse in each clinic, offering routine PMTCT care to HIV-infected pregnant women in the HIV clinics. Data for this study was collected using: 1) structured questionnaire (appendix II) to collect socio-demographic data, obstetric history, WHO staging and current CD4 count; 2) TB symptom and sign screen; 3) skin tests: PPD and TT antigen; 4) chest radiograph and 5) microbiology tests, sputum for microscopy (Ziehl-Neelsen staining**)** and culture for mycobacterium tuberculosis. Data collection tools are described in appendix II.

Research assistants were trained and mentored on the following skills: patient enrolment, testing algorithms; administration and reading PPD and TT antigen skin tests; procedures for sputum collection and handling and procedures for chest radiographs. The details of the standard operating procedures are described in appendix III.

2.5.2 Data Management

Data was entered daily into a Microsoft Access© data base. Double entry and logical syntaxes were performed to reduce errors of data entry. Data validation and cleaning was performed by pre-defined systematic post-entry computer checks: all missing values were listed, all values which were outside pre-defined range were listed, logical coherence checks was performed such as: inconsistent report between two fields and impossible differences between two fields for instance year of birth and age of the respondent and interpretation of skin test results. An attempt to get missing data and to correct the discrepancies was made before closure of the dataset.

2.6 Data Analysis and Presentation of Results

All patient names and identifiers were removed from all patient records before analysis. Only de-identified data was analyzed.

2.6.1 Descriptive Analysis: Data analysis begun with the summaries of demographic characteristics, patient's obstetric history, WHO stage and current CD4 count. These were presented descriptively in form of means or medians for continuous variables and proportions for categorical variables.

2.6.2 Primary Outcome Analysis: The prevalence of LTBI from those who tested positive to Mantoux Skin Test and the prevalence of presumptive active PTB based on chest radiographs suggestive of TB (all sputum samples were negative for ZN-staining and culture for *mycobacterium Tuberculosis*) were determined. Correlations between skin test result and: prior history of vaccination; WHO staging and CD4 counts were determined. It was not possible

to perform correlations with active TB because of small numbers of patients who had active TB in this study.

2.6.3 Secondary Outcome Analysis: The study was not powered to answer the secondary research objective. This limitation arose because of small numbers of those with presumed active TB as defined by chest radiographs and the fact that all microbiological test (Zn staining and culture) for mycobacterium tuberculosis were negative. These reasons made it difficult to determine the sensitivity, specificity and predictive values of the diagnostic test because of lack of power and a gold standard.

2.7 Ethical Considerations

This research was approved by the Moi Teaching and Referral Hospital/ Moi University School of Medicine Institutional Research and Ethics Committee (appendix VIII). HIV-infected pregnant women of reproductive age 15-49 years were enrolled. Eighteen is the age of consent according to the Kenyan law; however study participants between 15 and 18 years were regarded as emancipated minors since they were sexually active and pregnant thus gave consent on their own. There was little perceived risk to the patients in this study. No experimental therapies were given to patients; all patients who were diagnosed to have TB were managed according to the set protocols. Both Mantoux test and Tetanus Toxoid antigen tests are safe to use in pregnancy. Dose regulated single view PA chest radiographs are safe to use after the first trimester[59]. The principal investigator who has completed a Human Subjects Certification Course trained the research assistants on ethical issues including

confidentiality issues specific to this study. All patient paper records were kept in locked cabinets and electronic records within the database were password protected, and only data entry personnel and the principal investigator had access. In addition, only de-identified data was analyzed. Written informed consent was obtained in either English or Kiswahili (appendix VI and VII respectively) before the start of study. Patients were free to leave the study any time they felt uncomfortable without any penalties or loss of any benefits to which they were entitled to them in the health facility.

2.8 Study Limitations

This study was faced with a number of limitations: 1) none of the cases diagnosed with presumptive active TB on chest radiograph had microbiologic evidence of TB. Diagnosis was made based on radiological findings which are limited by the fact that it is not a gold standard. Studies powered to answer this question need to be developed, 2) loss-to-follow-up or missing data: 2 % of the study participants had some socio-demographic data missing; 7 % of the respondents did not come back for the reading of their skin tests; 10 % of the respondents who had a cough did not give their sputum samples (dry coughs) and 31 % did not get a chest radiograph done. Patients loss-to-follow up and missing data did not impact the studies power (with the exception of chest x-rays) because an additional 14 % of study participants above the calculated sample size were enrolled into the study. The reasons given for the missing chest radiographs included: patient declined, machine broke down, electricity black-out, and the study participants become lost to follow-up, 3) the study lacked a gold

standard to evaluate the secondary objective of sensitivity, specificity and predictive value of the various screening tests' and thus this study could not answer this question and 4) anergy panel has been shown to be more useful in patients with low CD4 counts, in this study; anergy panel with Tetanus Toxoid antigen was used for patients with no symptoms and signs regardless of their CD4 counts because the study did not control for CD4 counts.

CHAPTER 3: RESULTS

During the five month study period, a total of 190 patients who met the eligibility criteria and consented were enrolled and formed analysis for this study.

3.1 Descriptive results

The mean age in years of the study participants was 27 years with a median of 25 years and range of 15-43 years.

Table 1: Socio-demographic Variables

Variable	N (Freq %)
Marital status	
N	189
Married/ Living together	151 (80)
Separated	14 (7)
Single never married	13 (7)
Widowed	11 (6)
Employment	
N	190
Employed	34 (18)
School attendance	
N	190
Ever attended school	170 (89)
Education level	
N	170
College/ University	3 (2)
Secondary	36 (21)
Primary	131 (77)
Travel time to clinic	
N	187
More than 2 hours	13 (7)
Between 1 and 2 hours	29 (16)
Between 30 and 60 minutes	60 (32)
Less than 30 minutes	85 (45)

As shown in table 1 above, 80% of the study participants were married and living together, a small percentage (18%) of study participants was employed and a high percentage (89%) of them had some level of formal education.

Table 2: Obstetric History			
Variable	**N**	**Mean (std)**	**Median(range)**
Parity	188	2(1.87)	2(0,10)
Gravidity	188	3(1.86)	3(1, 11)
Gestation	184	26(7.18)	26(10, 41)
Number of children	189	2(1.66)	2(0, 10)

The mean and median (range) of: parity was 2 and 2(0, 10); gravidity was 3 and 3(1, 11); gestation in weeks was 26 and 26 (10, 41) and number of children was 2 and 2(0, 10) respectively (table 2).

Table 3: WHO Stage at Enrolment	
WHO Stage	**N=184 (Freq %)**
Stage 1	148 (80)
Stage 2	26 (14)
Stage 3	10 (6)
Stage 4	0 (0)

Study respondents at enrolment had a mean (std) CD4 count of 491 (279) cells/µl and median (range) CD4 count of 440 (24, 1408) cells/µl. Table 3, shows that at enrolment, a large proportion of the respondents (80%) were at WHO stage 1.

Table 4: Past Medical History

Variable	N	Yes N (Freq %)	No N (Freq %)	Unknown N (Freq %)
BCG Vaccination	190	164(86)	10(5)	16(8)
Tetanus Toxoid Vaccination	190	180(95)	9(5)	1(0.5)
Self History of TB	190	3(2)	186(98)	1 (0.5)
Family History of TB	190	12(6)	178(94)	0(0)

The respondents past medical history was as follows: 86% had BCG vaccination at infancy; 95% had tetanus toxoid vaccination at some point in their life; 2% gave a self history of past active tuberculosis and 6% had a family history of active tuberculosis (table 4).

Table 5 Symptom and Sign Screen at Enrolment		
Variable	**Yes** **N (Freq %)**	**No** **N (Freq %)**
Symptoms		
Cough	29 (15)	161 (85)
Fever	16 (9)	174 (91)
Night sweats	2 (1)	188 (99)
Weight Loss	29 (15)	161(85)
Signs		
Wasting	1 (1)	189 (99)
Abnormal chest exam	6 (3)	184 (97)

Table 5, gives a summary of the symptom and sign screen at enrolment. Out of the 15% of respondents who had a productive cough at enrolment, 21% had a cough for more than two weeks and 8% reported having blood stained sputum. The duration of fever in the 9% of respondents who had a fever at enrolment was: 75% of them for one week, 6% of them for two weeks, 6% of them for four weeks and 13% of them for more than four weeks. Only 1% of the study subjects reported drenching night sweats and 15 % reported having lost weight. On physical examination only 1 study respondent had wasting and 6 respondents had abnormal chest exam.

Table 6: Symptom/Sign Screen and Skin Test Summary			
Symptom/Sign Screen			
Variable	**N=187**		**Freq %**
Positive	38		20
Negative	149		80
Skin Tests			
Skin Test	**N**	**Positive N (Freq %)**	**Negative N (Freq %)**
Mantoux	167	65 (39)	102 (61)
Tetanus Toxoid	136	107 (79)	29 (21)

A small proportion (20%) of the respondents had a positive symptom and sign screen. On the other hand, mantoux skin test and tetanus toxoid skin test was positive in 39% and 79% of respondents respectively (table 6).

Table 7: Sputum Microbiology and Chest Radiograph Results				
Sputum Microbiology Results				
Variable	**N**	**Negative for TB N: freq (%)**	**Positive for TB N: freq (%)**	**Missing sample N: freq (%)**
Microscopy (ZN-staining)	28	25(89)	0	3(11)
Culture	28	25(89)	0	3(11)
Chest Radiograph Results- Suggestive or not Suggestive of TB				
Variable	**N**	**Suggestive of TB N (Freq %)**	**Not Suggestive of TB N (Freq %)**	
Clinician read	127	5 (4)	122 (96)	
Radiologist read	128	5 (4**)**	123 (96)	

None of the 28 samples of sputum's collected were positive for ZN staining or mycobacterium tuberculosis culture. Chest radiograph results suggestive of active TB were 5 among clinician read and 5 among radiologist read chest radiographs. There was no agreement between the clinician and radiologist read chest radiographs for those that were suggestive of active TB. This means that the clinician and radiologist read chest radiographs suggestive of TB were totally different from each other (table 7).

Table 8: Actual Report of Chest Radiographs

Variable	N	Normal N:Freq (%)	Cavity N:Freq (%)	Milliary N:Freq (%)	Infiltrate N:Freq (%)	Poor Quality N:Freq (%)	Other N: Freq(%)	Missing N: Freq (%)
Clinician Read	106	62(58)	1(1)	0 (0)	32(30)	4(4)	7(7)	84
Radiologist Read	130	74(57)	1(1)	3(2)	24(19)	12(9)	16(12)	60

There was a significant proportion of chest radiographs reported as abnormal but not suggestive of active TB. Of note is the high proportion of chest radiographs reported to have infiltrates; 30% among clinician read and 19% among radiologist read (Table 8) this are potential cases of active TB. It is noted that there are many missing results on this table. This is because the actual chest radiograph readings were incidental findings; the study was designed to answer if the radiograph was suggestive or not suggestive of TB but the actual report was also written in many of the reports.

3.2 Prevalence of Latent TB Infection

Table 9: Skin Tests-Mantoux and Tetanus Toxoid			
	Tetanus Toxoid (TT)		
Mantoux	Positive	Negative	**Total**
Positive	48	5	**53**
Negative	57	24	**81**
Total	**105**	**29**	**134**

Of the study participants, 134 of them had results for both Mantoux and Tetanus Toxoid skin tests (table 9). Computations of table 9 are as follows:

3.2.1 Prevalence of Latent TB Infection (LTBI)

Positive Mantoux regardless of TT result = LTBI

53/134 =40 %

3.2.2 Prevalence of True Negative mantoux reaction

Negative mantoux with Positive TT = True negative mantoux= No latent TB infection

57/134=43%

3.2.3 Prevalence of Anergy

Negative mantoux with Negative TT = Anergy

24/134=18%

From table 9 computations: the prevalence of latent TB infection, true negative mantoux reaction and anergy is: 40%; 43% and 18% respectively.

3.3 Prevalence of Presumptive Active Pulmonary Tuberculosis (PTB)

Table 10: Prevalence of Presumptive Active TB

Chest radiograph	**Microbiology result for *M. Tuberculosis* ***			
	Positive	Negative	No sputum (dry cough)	**Total**
Suggestive of TB	0	3	7	**10**
Not suggestive of TB	0	22	96	**118**
Missing	0	3	59	**62**
Total	**0**	**28**	**162**	**190**

* *(both Zn staining and Culture)*

The prevalence of TB will be presumed in this study because all the sputum samples taken for both microbiology (ZN staining and culture) were negative for mycobacterium tuberculosis. The prevalence of presumptive active TB in this study is 5%. This was arrived at based on clinician and radiologist read chest radiographs that were suggestive of TB (all the 10 patients found to have chest radiographs suggestive of TB were commenced on anti-tuberculosis treatment according to the set protocols). The denominator used for prevalence of TB is 190-the total number of patients enrolled in the study. It is notable that 7 out of 10 patients who had presumptive TB had dry cough (table 10).

Table: 11 Mantoux, Cough and Chest Radiograph Report for those with Presumptive Active TB			
Mantoux	**Positive**	**Negative**	
N	4	6	
Cough	**Yes**	**No**	
N	3	7	
Chest radiograph Report	**Milliary**	**cavity**	**Infiltrate**
N	3	2	5

Table 11 shows the Mantoux, cough status and chest radiograph report for the 10 patients with presumptive active TB. Mantoux was positive for 4 patients and 3 patients had a cough. The radiology report for the 10 patients was as follows: 3 milliary picture, 2 cavitary disease and 5 infiltrates.

3.4 Correlations

Table 12: Correlation between Prior History of Vaccination and Skin Test Result			
		Skin Test Result	
Prior History of Vaccination (Skin Test Administered)	**N**	**Positive N: (Freq %)**	**Negative N: (Freq %)**
Tetanus Toxoid (Tetanus Toxoid Skin test)	126	99 (79)	27 (21)
BCG (Mantoux Skin Test)	143	57 (40)	86 (60)

Of the study subjects who reported to have had tetanus vaccination, 79% had a positive tetanus toxoid result. On the other hand, of the study subjects who

reported to have had BCG vaccination, 40% had a positive Mantoux skin test result (table 12).

Table 13: Correlation between WHO Staging and Skin Tests

WHO Staging	Positive Mantoux N: (Freq %)	Positive TT N: (Freq %)	Totals
Stage 1	47 (75)	85 (83)	**132**
Stage 2	11 (18)	14 (14)	**25**
Stage 3	5 (8)	4 (4)	**9**
Stage 4	0(0)	0(0)	**0**
Totals	**63**	**103**	**166**

Correlation of WHO staging with positive mantoux skin test were as follows: 75% stage 1; 18% stage 2, 8% stage 3 and none stage 4. On the other hand, correlations with positive tetanus toxoid skin test were as follows: 83% stage 1; 14% stage 2, 4% stage 3 and none stage 4 (table 13).

Table 14: Correlation between CD4 Counts and Skin Tests

Skin Test Result	CD4 Count in cell/µl	
	Median	**Range**
Positive Mantoux	399	36-1408
Negative Mantoux	462	24-1241
Positive Tetanus Toxoid	470	24-1408
Negative Tetanus Toxoid	403	15-1218

Table 14 shows correlations between CD4 counts and skin tests. High median CD4 counts were observed in all categories of skin test results. The median (range) CD4 counts in cells/µl were as follows for those who had a: positive mantoux test 399 (36-1408); negative mantoux test 462 (24-1241); positive tetanus toxoid test 470 (24-1408) and negative tetanus toxoid test 403 (15-1218).

3.5 Sensitivity, Specificity and Predictive Value of the Diagnostic Tests

It was not possible to compute the sensitivity, specificity and predictive values for the diagnostic tests because of small numbers (only 10) of study participants with presumed active TB disease and all had their sputum's negative for mycobacterium tuberculosis hence no gold standard.

CHAPTER 4: DISCUSSION

The study population was made up of HIV-infected pregnant women being cared for within the AMPATH program. The prevalence of Latent TB Infection (LTBI) was 40% and that of presumptive active TB was 5% (based on chest radiograph results that were suggestive of TB). Dose regulated chest radiographs after the first trimester play an important role in the screening and diagnosis of active TB among HIV-infected pregnant women. However screening and diagnosis of TB among this group of patients still remains a big challenge.

The prevalence of LTBI is high (40%) in this study population. In addition 43% of the study population were true negatives for mantoux skin test and only 18% had anergy. This finding is significant because, if active TB can be ruled out, potentially more than 80% of them are eligible for Isoniazid Preventive Therapy (IPT) which is the treatment for LTBI. Treating LTBI among HIV-infected pregnant women is important because unlike their non-infected counterparts they are at a higher risk (5-15% annually) of reactivation TB [45].

IPT is safe to use in pregnancy[64] and can be co-administered together with combined anti retroviral therapy (cART) given for purposes of PMTCT. The PMTCT program enjoys a well established lost-to-follow-up and adherence program in most set-ups. In addition cART for PMTCT starts from 14 weeks gestation to 1 week after breastfeeding for those not otherwise eligible for cART and for life for those eligible for cART[65]; thus this patient population is already

"captured" within a care system with well designed follow-up and adherence interventions. It is also a perfect time for concomitant IPT to be given without an added burden on the patient for additional appointments or the health care system for resources and is enough time to complete a 9 month IPT regimen if started during the antenatal period. For this reason IPT programs among HIV-infected pregnant women should be integrated into already well established PMTC programs for it to be feasible, scalable and sustainable in our resource limited settings with high TB and HIV burden.

Antepartum IPT programs integrated on prenatal care programs are a reasonable approach to TB prevention services in settings where postpartum adherence and loss to follow-up is high. In resource constrain settings the antenatal care period is often the only contact that many high-risk women such as HIV-infected pregnant women have with the health care system[66]. Pregnancy presents a unique opportunity to provide monitored isoniazid treatment, and community services are maximized, decreasing socio-economic barriers to care. Scheduled visits occur frequently antenatally and this will help facilitate monitoring for compliance and isoniazid-induced hepatotoxicity[64]. Delay of therapy until the postpartum period can incur unnecessary risk, because a significant proportion of pregnant women with LTBI might not return to receive IPT[6, 64].

The prevalence of presumed active TB in this study population (5%) is lower than what is estimated by the Kenya Ministry of Health Division of Reproductive Health (12.5%)[34] but higher than that documented in Durban, South Africa 0.1% and 0.6% in 1996 and 1998 respectively among pregnant women [11]. This prevalence is likely an under estimate because more than 40% of women had abnormal chest radiographs that may have been active TB if further workup or longer follow-up was carried out. This prevalence however, is sufficient to call for the development of a structured, systematic screening of TB among HIV-infected pregnant women in areas of high TB burden. Undiagnosed active TB in this group of patients may lead to untoward effects in the following ways: with the current PMTCT recommendations of using cART from 14 weeks gestation[65] there is a risk of fatal Immune Reconstitution Inflammatory Syndrome (IRIS) occurring in cases of undiagnosed active TB[24-31]; maternal mortality[13-20]; preterm delivery[23] and risk of TB transmission to the: foetus, newborn, obstetric ward, family and community at large[40].

This study has been able to highlight three main screening and diagnostic challenges of TB faced in a HIV-infected pregnant population. The first challenge is with regard to the fact that there is no gold standard for LTBI. Attempts to define “anergy” and verify the true negative nature of a “negative PPD” have long been debated. This study attempted to use TT to verify that a negative PPD was truly representative of no TB infection. Anergy was defined by this study as a negative TT and a negative PPD. A noteworthy proportion (18 %) of patients

was anergic and their true TB infection status is not known. This may not be surprising as all of patients in this study had a reason to have a poorly functioning immune system- that is their HIV infection. The high rate of positive PPD (40%) coupled with the reasonably high rate of possible anergy (18%) raises the question of whether strategies that omit PPD as entry criteria for IPT programs are a reasonable strategy. It is mandatory to rule out active TB prior to initiation of IPT but this can be performed by a negative: symptom screen, physical exam, and chest radiograph. This is in fact the protocol that is utilized in the non pregnant population of AMPATH. Further studies are needed to define the incidence of TB and HIV disease in populations where use of the PPD for stratification and enrolment in IPT studies are necessary like in low TB burden settings. In settings such as described in this study with a high TB and HIV burden it may be reasonable to remove PPD screening prior to IPT. It appears reasonable in our pregnant population to at least begin with a protocol that all HIV-infected pregnant women have a dose regulated PA chest radiograph after the first trimester as standard of care to rule out active PTB and subsequently give IPT because a positive PPD should be followed by a chest radiograph in all cases to rule out active PTB before IPT is commenced.

The second challenge is with the limitations and challenges of TB microbiological diagnosis which is underscored in this study. In this study, 20% of the participants had positive symptoms and sign screen and none of the sputum samples taken for microbiology demonstrated *M. Tuberculosis* on ZN staining or

culture. This means that patients with military disease, cavities or abnormal infiltrates found because the radiographs were performed by algorithm rather than symptoms were likely early in the TB disease process and could have been missed out. It's known that the earlier the disease, the lower the organism burden and the higher the chance of either or both negative smears and cultures. This study was not powered to address "best" diagnostic practices and thus recommendations on how to screen for active disease cannot be made from this study. It can only be noted that a significant number of patients were found by chest radiographs in this selected population.

The third challenge brought out in this study is in relation to the fact that diagnosis of presumptive active TB was based on chest radiograph results because the microbiology results were all negative. Chest radiography is not the gold standard for diagnosis of TB but one of the tools that aids diagnosis. Of note, was the fact that there was no agreement between radiographers and clinicians. Of concern however, are the three "classic' miliary TB cases which were missed by clinicians. Thus, if radiography is to be used in the algorithm of diagnosis for these patients training of the front line clinicians in all TB patterns is necessary. Initial interpretation of the chest radiograph is usually done by the clinician in the clinics, who in resource constrained setting like in this study are usually clinical officers (mid-level practitioners) for urgent diagnosis and management. Turn-around time for radiologist read chest radiographs take a long time and radiologist may not be available in most cases. For chest

radiographs to have an impact, clinicians in the PMTCT clinic must be trained and mentored on how to interpret chest radiographs for purposes of diagnosing PTB.

This study was faced with a number of limitations: the diagnostic challenge; loss-to-follow-up and lack of power to determine the secondary objective of sensitivity, specificity and predictive value of the screening tests. Regardless of these limitations the primary research questions were adequately answered and information gained on this will be used as pilot data to generate hypothesis for future studies in this field. These results can be generalized to HIV-infected pregnant women in resource limited settings with a high HIV and TB burden as well.

Conclusion

The prevalence of LTBI is high in pregnant women attending PMTCT Clinics in Western Kenya. The prevalence of presumed TB disease diagnosed following an algorithm of shielded radiographic criteria was significant with a notable milliary presentation.

Recommendations

Recommendations based on this study are: TB screening should be routine in all PMTCT care programs and more studies are needed to determine best screening protocols as well as feasibility of IPT among HIV-infected pregnant women in resource constraint high TB and HIV burden settings.

CHAPTER 5: APPENDIXES

Appendix 1: References

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Appendix II: Structured Questionnaire

Screening of pulmonary tuberculosis, in HIV infected pregnant women in Western Kenya

Serial Number…….

Name……. Phone No…… Clinic…… Date…….

Eligibility criteria

Inclusion criteria

1) In reproductive age, 15-49 years
2) HIV-infected pregnant women newly enrolled to the pMTCT program
3) ARV naïve
4) Patients who are able to return to the clinic after 48 hours for the reading of mantoux test antigen and collection of 2nd and 3rd sputum samples
5) Written or verbal informed consent

Exclusion criteria

1) Patients on TB treatment at the time of enrollment
2) Patients with a past history of Isoniazid Preventive Therapy (IPT)
3) Patients who present in labor

A) Questions to study participants

1. **Is the patient eligible for the study?** Yes □ No □

(Kiswahili version is in italics after the English version)

2. Year of Birth (*Mwaka wa kuzaliwa* **) ……**

3. Age in years (*umri wako***) ……..**

4. How long did it take you to travel to clinic today?

(*Ilikuchukua muda kiasi gani kusafiri hadi kwenye kliniki leo?)*

Less than 30 minutes (*Chini ya dakika 30)* □

Between 30 and 60 minutes (*kati ya dakika 30 na 60)* □

Between 1 and 2 hours (*kati ya saa moja na masaa mawili)* □

More than 2 hours (*zaidi ya masaa mawili)* □

5. What is your current marital status?

(Hali yako ya kiuhusiano ikoje?)

Married/ Living together (*katika ndoa/Kuishi pamoja)* □

Single never married (*hujawahi kuolewa)* □

Separated (*mmetengana*) □

Divorced (*mmetaliki)* □

Widowed (*mjane)* □

6. How many children do you have? (*Je, una watoto wangapi?)*

.....

7. Have you ever attended school? (*Je, wewe umepata elimu yoyote?)*

Yes (*Ndio)* □

No (*La)* □

8. If YES, to question 7 above what level of education do you have?

(*Iwapo jibu lako katika swali la 12 ni NDIO, eleza kiwango cha elimu yako)*

Primary (*Shule ya msingi)* □ *Class ……...*

Secondary (*shule ya upili)* □ Form……

College/university (*chuo kikuu/taasisi ya elimu ya juu)* □

9. Are you employed (*Je, umeajiriwa kazi?)*

Yes (*Ndio)* □

No (*La)* □

10. If YES, to question 9 above what type of employment do you have?

(Ikiwa jibu lako katika swali la 14 ni NDIO, eleza unafanya kazi ya aina gani?)

Self Employed *(Ninafanya kazi yangu binafsi)* □

Paid employment (*Nimeajiriwa)* □

B) From Patients chats

11. Parity ……

12. Gravida …….....

13. Gestation By Date (GBD) in weeks ……

14. Lowest CD4 count ……

15. Latest CD4 count……

16. WHO stage……

C) Clinical, Laboratory and Radiological Investigations

Has the patient completed the structured questionnaire? *Yes* □ *No* □

*If **YES** to above question proceed to question 1*

*If **NO** kindly complete the structured questionnaire first before proceeding to question 1*

I) SYMPTOM SCREEN *(MAJARIBIO YA KUTAMBUA DALILI)*

1. **Did you get BCG vaccination when you were a baby?**

   (*Je, ulipata chanjo ya BCG ulipokuwa mtoto?)* **[check scar to confirm]**

   Yes *(Ndio)* □

   No *(La)* □

   Unknown *(Sijui)* □

2. **Have you ever been given Tetanus Toxoid vaccination?**

   (Je, umeshawahi kupata chanjo ya pepopunda?)

Yes *(Ndio)* □

No *(La)* □

Unknown *(Sijui)* □

3. **Have you ever been told that you have tuberculosis?**

   (Umeshawahi kuambiwa kwamba unaugua ugonjwa wa kifua kikuu?)

   Yes *(Ndio)* □

   No *(La)* □

4. **If YES to question 3 above were you treated for tuberculosis?**

   (Iwapo jibu lako ni NDIO katika swali la 3, je ulipata matibabu?)

   Yes *(Ndio)* □

   No *(La)* □

5. **If YES to question 4 above did you complete your medication?**

   (Iwapo jibu lako katika swali la 4 ni NDIO, je, ulikamilisha kumeza zile dawa ulizopewa?)

   Yes *(Ndio)* □

   No *(La)* □

6. **If YES to 5 above, how many months of treatment?**

   (Iwapo jibu lako katika swali la 5 ni NDIO, ulizimeza zile dawa kwa muda wa miezi mingapi?)

…….

7. **Has anyone in your family (living in your household) ever been told that they had tuberculosis?**

(Je, kuna mtu yeyote katika familia yako (mnayeishi naye) ambaye ameshawahi kuugua ugonjwa huu wa kifua kikuu?)

Yes *(Ndio)* □

No *(La)* □

8. **Do you have a cough?**

   (Je, unakohoa?)

   Yes *(Ndio)* □

   No *(La)* □

9. **If YES to question 8 above has it lasted more than two weeks?**

   (Iwapo jibu lako katika swali la 8 ni NDIO, je,kukohoa huko kumekuwapo kwa muda wa zaidi ya wiki mbili?)

   Yes *(Ndio)* □

   No *(Yes)* □

10. **If YES to question 9 above are you coughing blood?**

    (Ikiwa jibu lako katika swali la 9 ni NDIO, je, umekohoa kikohozi kilicho na damu?)

    Yes *(Ndio)* □

    No *(La)* □

11. **Do you have a fever?**

    (Je, una homa? Au tatizo la kuwa na joto jingi?)

    Yes *(Ndio)* □

    No *(La)* □

12. **If yes to question 11above for how long have you had fever?**

(Ikiwa jibu lako katika swali la 11 ni NDIO, eleza muda ambao umekuwa na homa hii?)

1 week *(Wiki 1)* □

2 weeks *(Wiki 2)* □

3 weeks *(Wiki 3)* □

4 weeks *(Wiki 4)* □

More than 4 weeks *(Zaidi ya wiki 4)* □

13. Do you have drenching (soaking) night sweats?

(Je, una tatizo la kutokwa na jasho sana wakati wa usiku?)

Yes *(Ndio)* □

No *(La)* □

14. **Have you lost weight or failed to gain weight in this current pregnancy?**

(Je, umepoteza au kukosa kuongeza uzito katika hali hii yako ya ujauzito?)

Yes *(Ndio)* □

No *(La)* □

Weight now.....

Previous visit, weight prior to pregnancy .....

II) SIGN SCREEN (PHYSICAL EXAM)

1. General condition

Good **(Normal)** □

Wasting **(Abnormal)** □

2. **Vital signs (normal ranges in brackets)**

   a) Temperature……^{0}C (normal up to 37.2^{0} C)

   Normal □ **Abnormal** □

   b) Respiratory rate…….. (Normal up to 20/minute)

   Normal □ **Abnormal** □

   c) Oxygen saturation……..% (normal more than 92%)

   Normal □ **Abnormal** □

3. **Chest exam**

   Normal □

   Abnormal □

4. **If abnormal to question 3 above, what are the examination findings?**

Percussion: Dullness □

Auscultation: Breath sounds diminished □ Bronchial breath sounds □

Rhonchi □ Crepitations □

- ***YES*** *to any of the responses in section A above (symptom screen) and or* ***Abnormal*** *to any of the responses in section B above equals a* ***POSITIVE*** *symptom/sign screen*
- ***NO*** *to any of the responses in section A above (symptom screen) and or* ***NORMAL*** *to any of the responses in section B above equals a* ***NEGATIVE*** *symptom/sign screen*

III) MANTOUX AND TETANUS TOXOID ANTIGEN SKIN PLANTING

All patients with a positive symptom/sign screen: administer mantoux antigen only, answer question 1- 3 (patients with negative symptoms /signs move to question 4)

1. Study participant with Positive symptom/sign screen

   Yes □ No □

2. If yes to 1 above did you administer mantoux

   Yes □ No □

3. If No to 2 above why?

   Patient declined □ Mantoux not available □

   Other **……**

All study participants with a negative symptom/sign screen: administer both mantoux and tetanus toxoid antigen answer question 4-6

4. Study participant with Negative symptom/sign screen

   Yes □ No □

5. If yes to 4 above did you administer both mantoux and tetanus toxoid antigen

   Yes □ No □

6. If No to 5 above why?

   Patient declined □ Mantoux and or tetanus toxoid not available □ other …

IV) MANTOUX AND TETANUS TOXOID ANTIGEN RESULS

Using the pen method, measure the induration on the volar region of the arm in millimeters (mm) after 48 hours and record below: Test result more than 5 mm is positive

Measurement in mm …….

0-4 mm □

≥ 5mm □

Mantoux Positive □ Negative □

TT antigen Positive □ Negative □

V) CHEST RADIOGRAPHS

All study participants will undergo a dose regulated shielded PA chest radiograph after the 1st trimester

1. Has the patient undergone a dose regulated shielded PA chest radiograph after the 3rd trimester?

   Yes □ No □

2. If No to 1 above why?

   Patient declined □ Chest radiograph machine not working □

   Radiographer not available □

   Other…….

3. Radiology report by clinician in the clinic:

……

……

……

Not suggestive of TB □ **Suggestive of TB** □

4. Radiology report by radiologist:

……

……

……

Not suggestive of TB □ **Suggestive of TB** □

VI) SPUTUM MICROSCOPY AND CULTURE

Collect 3 sputum samples from those patients with productive cough

- *Sample 1-first spot sample on first contact with the patient*
- *Sample 2- second sample will be collected early morning after 48 hours (same day of reading the skin tests)*
- *Sample 3- on return to the clinic*

1. ***Sputum for microscopy (***Ziehl-Neelsen ***[ZN]*** *staining****) result:***

Laboratory

report……

……

……....

Sputum positive for ZN staining means Positive for TB

Sputum negative for ZN staining means Negative for TB

Positive for TB □ Negative for TB □

2. ***Sputum for culture result:***

Laboratory report

……

……

……

Culture positive for mycobacterium tuberculosis means Positive for TB

Culture negative for mycobacterium tuberculosis means Negative for TB

Positive for TB □ Negative for TB □

Appendix III: Standard Operating Procedures

Standard Operating Procedure for enrolment and testing algorithm

1. Patients meeting the set criteria will be asked to give a written informed consent before being enrolled to the study, in the PMTCT HIV clinic by the research assistants.
2. A pre-tested structured questionnaire will be completed for all enrolled patients by clinical officer.
3. A pre-tested standardized symptom/sign screen will be completed for all enrolled patients by the clinical officer.
4. Patients with positive symptoms and or signs:
    i. Will be administered mantoux test by the nurse
    ii. Will be asked to undergo a single view PA, dose regulated chest radiograph by the clinical officer
    iii. Will be asked to produce sputum for microscopy (Ziehl-Neelsen staining) and culture for mycobacterium tuberculosis by clinical officer
5. Patients with negative symptoms and signs:
    i. Will be administered both mantoux test and TT antigen by the nurse
    ii. They will be asked to undergo a single view PA, dose regulated chest radiograph by the clinical officer
6. Patients who are diagnosed to have active pulmonary TB at any stage of the testing algorithm will be treated according to the DLTLD national program protocol in the PMTCT clinic

Standard Operating Procedure for Tuberculin PPD (Mantoux) Test

All eligible patients will be administered mantoux antigen. Mantoux test produces a type IV delayed cell mediated hypersensitivity reaction and is an accepted aid in the diagnosis of tuberculosis. The reaction to intra-dermally injected tuberculin reaches its peak in 48-72 hours after administration and consists of induration caused by cellular infiltration of lymphocytes. Mantoux is ordinarily used for testing presence of tuberculosis. Mantoux test is safe to use in pregnancy and adverse effects rarely occur, this include: a) local adverse effects: vesiculation, ulceration or necrosis may appear at the test site, b) systemic adverse effects: immediate or generalized skin rash very rarely occurs.

The following is the operating procedure for this test:

1. Epinephrine Hydrochloride Solution (1:1,000), antihistamine and steroids should be readily available for immediate use in case an anaphylactic or acute hypersensitivity reaction occurs (this very rarely occurs).
2. The site of the test, is the flexor surface of the left forearm about 4 inches below the bend of the elbow (volar surface), any visible blood vessel should be avoided.
3. The skin of the forearm is first cleansed with alcohol swab and allowed to dry.
4. Disposable sterile syringes and needles will be used.

5. The rubber cap of the vial should be wiped with cotton moistened with alcohol. The needle is then inserted gently through the cap and the required amount of Tuberculin PPD is drawn into the syringe.
6. The test dose 5 TU per test dose of 0.1 mL of Tuberculin PPD (mantoux) is administered with a 1 mL syringe calibrated in tenths and fitted with a short, one-half inch 26 or 27 gauge needle. PPD must be drawn up immediately before use and not in batches pre-made due to loss of potency as the tuberculin can adhere on to plastic syringes over time.
7. The point of the needle is inserted into the most superficial layers of the skin with the needle bevel pointing upward. Once underneath the skin, the needle bevel is rotated 180 degrees prior to injection. If the intra-cutaneous injection is performed properly, a definite bleb will rise at the needle point, about 10 mm (3/8") in diameter. This will disappear within minutes. No dressing is required.
8. A separate sterile syringe and needle must be used for each individual injection to prevent the possibility of transmission of viral hepatitis or other infectious agents from one person to another.
9. Store Mantoux test at 2º to 8ºc (35º to 46ºf) and do not freeze. Discard product if exposed to freezing. Tuberculin solutions can be adversely affected by exposure to light hence should be stored in the dark except when doses are actually being withdrawn from the vial. A vial of tuberculin which has been entered and in use for 30 days should be discarded because oxidation and degradation may have reduced the potency.

10. Ask patients to come back to the clinic in 48 hours and to report any of the adverse effects mentioned above.
11. Universal precautions of infection prevention should be observed by the health care provider at all times.
12. The site of the PPD will be recorded on the patient's encounter form to avoid confusion at time of reading.

Interpretation of the mantoux Test

A positive reaction indicates sensitivity to tuberculin, which may be the result of a previous infection with mycobacteria. The test should be read 48 to 72 hours after administration of the tuberculin. For purposes of this study 48 hours will be used to give a grace period of 24 hours for patients who might miss their appointments. Sensitivity is indicated by induration, usually but not always accompanied by erythema. The pen method of reading will be utilized to minimize inter-reader variability. The widest diameter of distinctly palpable induration should be recorded in millimeters (mm) using a plastic ruler. An induration of ≥ 5 mm will be classified as positive in this group of patients.

Standard Operating Procedure for Tetanus Toxoid (***TT) antigen Test***

Tetanus immunization is usually combined with diphtheria and pertussis immunization (DPT), given alone to pregnant mothers as part of their antenatal care profile, routinely given after injuries with a break to the skin barrier in emergency rooms in Kenya and tetanus spores are wide spread in the environment. TT antigen causes a type IV delayed cell mediated hypersensitivity

reaction. The reaction to intra-dermally injected TT antigen reaches its peak in 48-72 hours after administration and consists of induration caused by cellular infiltration of lymphocytes. TT antigen is safe to use in pregnancy and adverse effects rarely occur. Local adverse effects: redness, warmth, and edema at injection and very rarely anaphylactic reaction have been reported.

The following is the operating procedure for this test:

1. Epinephrine Hydrochloride Solution (1:1,000), antihistamine and steroids should be readily available for immediate use in case an anaphylactic or acute hypersensitivity reaction occurs.
2. Dilute TT antigen test dose before use. For 100 doses that will need to be used within 4 hours - Dilute the one (1) ml. 10 dose vial with 10 ml of sterile normal saline. This gives a 1:10 dilution. For smaller number of doses, draw a set volume (1-10 ml) of normal saline into a sterile 20 ml syringe and follow with a volume of the tetanus toxoid antigen (0.1-1.0 ml) that equals 1/10 of the volume of normal saline to make the 1:10 dilution.
3. The site of the test, is the flexor surface of the Right forearm about 4 inches below the bend of the elbow (volar surface), any visible blood vessel should be avoided.
4. The skin of the forearm is first cleansed with alcohol swab and allowed to dry.
5. Disposable sterile syringes and needles will be used.

6. The rubber cap of the vial should be wiped with cotton moistened with alcohol. The needle is then inserted gently through the cap and the required amount of candin is drawn into the syringe.
7. The test dose (0. 1 mL) of 1:10 dilution TT antigen is administered with a 1 mL syringe calibrated in tenths and fitted with a short, one-half inch 26 or 27 gauge needle.
8. The point of the needle is inserted into the most superficial layers of the skin with the needle bevel pointing upward. Once underneath the skin, the needle bevel is rotated 180 degrees prior to injection. If the intra-cutaneous injection is performed properly, a definite bleb will rise at the needle point, about 10 mm (3/8") in diameter. This will disappear within minutes. No dressing is required.
9. A separate sterile syringe and needle must be used for each individual injection to prevent the possibility of transmission of viral hepatitis or other infectious agents from one person to another.
10. Store TT antigen at 2º to 8ºc (35º to 46ºf) and do not freeze. Discard product if exposed to freezing.
11. Failure to store and handle TT antigen as recommended will result in a loss of potency and inaccurate test results.
12. Universal precautions of infection prevention should be observed by the health care provider at all times.
13. The site of the TT injection will be recorded on the patient's encounter form to avoid confusion at time of reading.

Interpretation of the TT Antigen Test

A positive reaction indicates sensitivity to TT antigen, which may be the result of a previous vaccination or infection. The test should be read 48 to 72 hours after administration of TT. For purposes of this study 48 hours will be used to give a grace period of 24 hours for patients who might miss their appointments. Sensitivity is indicated by induration, usually accompanied by erythema. The widest diameter of distinctly palpable induration should be recorded in millimeters (mm) using a plastic ruler. An induration of ≥ 5 mm is will be classified as positive in this group of patients.

Standard Operating Procedure for sputum microscopy (Ziehl-Neelsen staining) and culture for mycobacterium tuberculosis

Patients, who are positive for symptom/sign screen and can produce sputum, will be asked to give sputum samples. Sputum samples for this study will be collected as follows: the first spot sample on first contact with the patient, second sample will be collected early morning after 48 hours (when the patient will be coming for the skin tests readings) and a third spot specimen on return to the clinic. Sputum from each patient will be taken to the laboratory for smear microscopy and culture. All specimens will be refrigerated and transported to the Mycobacteria Reference Laboratory at Eldoret AMPATH clinic for microscopy and culture to ensure there is standardization.

Standard Operating Procedure for chest radiographs

All patients participating in this study will be asked to do a single view dose regulated shielded PA chest radiograph. A consultant radiologist will visit the study sites, to ensure that the chest radiograph machines are the recommended ones with appropriate shielding and to standardize the procedure. The following is the operating procedure for chest radiographs:

1. They will be done after first trimester when the risk of teratogenicity has been shown to be low unless otherwise indicated by the clinician seeing the patient
2. Full size chest radiographs; which are recommended in pregnancy because of low dose skin entrance of radiation of about 20mrad will be used in this study, as opposed to mass miniature chest radiography with high dose skin entrance of radiation of about 400-600mrad which will not be used in this study
3. Appropriate shielding will be provided
4. The chest radiographs will be interpreted by clinicians on site for care but by radiologists for the study purposes
5. Results of the readings by clinicians will be compared to that of the radiologist. The radiologist readings will be made available to the clinician caring for the patient

Appendix IV: Time Frame

Activity	January-February 2009	March - August 2009	September 2009	October 2009- March 2010	April 2010	May-June 2010
Proposal Development						
Ethical approval and Seeking for funding						
Planning and set up						
Data collection						
Data analysis						
Report writing Presentation of Results						

Appendix V: Budget

ITEM	QUANTITY	UNIT COST (KSH)	TOTAL COST (KSH)
PROPOSAL DEVELOPMENT			
Printing paper	3 Reams	360	1,080
Printing	1500 Sheets	5	7,500
Binding- soft Cover	6 Copies	100	600
Sub-Total			**9,180**
PERSONNEL			
Clinical officers (for 4 months)	6	2,000 per month	48,000
Nurses (for 4 months)	3	2,000 per month	24,000
Biostatistician	1	50,000	50,000
Sub-Total			**122,000**
TRAINING OF RESEARCH ASSISTANTS			
Note books	10	50	500
Ball Point pens	1 pack	180	180
Pocket files	10	50	500
stapler	1	200	200
Staples	1 Packs	200	200
Paper Punch	1	400	400
Permanent Marker pens	1 Pack	400	400
Sub-Total			**2,380**
PRE-TESTING OF TOOLS			
Printing Paper	1 Ream	360	360
Printing	500 Sheets	5	2,500
Pocket files	10	50	500
Mantoux Antigen	10 doses	800	8,000
Tetanus Toxoid Antigen	10 doses	50	500
Permanent Marker pens	1 Pack	400	400
1cc syringes calibrated in tenths	60	2	120
One-half inch 26 or 27 Gauge needles	60	2	120
Gloves	3 Boxes	170	510
Cotton Wool	3 Rolls	200	600
Methylated Spirit	3 Liters	200	600
Sub-Total			**14,210**
DATA COLLECTION			
Printing Paper	10 Reams	360	3,600
Printing	40 sheets	5	200
Photocopying	4,000 Sheets	1	4,000
Cabinets	2	3,000	6,000
File folders	200	50	10,000
Suspender Files	50	50	2,500
1 cc Syringes calibrated in tenths	400	2	800
One-half inch 26 or 27 Gauge needles	400	2	800

Gloves	10 Boxes	170	1,700
Methylated Spirit	15 liters	200	3,000
Permanent Marker pens	3 Packs	400	1,200
Tape Measures	10	20	200
Mantoux Antigen	200 Doses	800	160,000
Tetanus Toxoid Antigen	100 Doses	50	5,000
Chest Radiographs	200	400	80,000
Sputum Cultures	100	800	80,000
Transport for Patients	300 visits	300	90,000
Sub-Total			**369,000**
FINAL REPORT			
Printing Paper	2 Reams	360	720
Printing (sheets)	1000	5	5,000
Binding- Hard Cover	6 Copies	500	3000
Sub-Total			**8,720**
Total			**525,490**

Appendix VI: Informed Consent (English)

Consent Statement

This study seeks to find out the prevalence of latent and active pulmonary tuberculosis in HIV infected pregnant women in Western Kenya and the role of clinical, laboratory and radiological investigations in diagnosing active pulmonary TB in HIV infected pregnant women.

Study Purpose

You are invited to participate in a research study conducted by investigators from University of Nairobi School of Medicine, to find out the role of symptom/sign screen, Mantoux test, Tetanus Toxoid (TT) antigen, single view dose regulated shielded chest radiographs, sputum smear and sputum cultures in diagnosing active pulmonary tuberculosis (TB) in HIV infected pregnant women in AMPATH.

TB is the most common opportunistic infection affecting HIV/AIDS patients. Diagnosis of TB in pregnancy may be delayed by the non-specific nature of early symptoms and the frequency of malaise and fatigue in pregnancy, similar to early symptoms of TB. Untreated TB can endanger yourself or the baby. In addition, as part of your care, antiretrovirals to prevent transmission of the HIV virus to your unborn child will be given to you; it is very important that TB, if present, is diagnosed before start of these drugs. It is for this reason that the researchers would like to find out the best practices in diagnosing TB in HIV infected pregnant women.

Number of People Taking Part in the Study

If you agree to participate, you will be one of 183 other pregnant women on follow up at the Eldoret and Busia AMPATH clinics who will participate in this study

Procedure for the Study

If you agree to be part of the study and you will be asked to do the following:

1. You will be assisted by our trained research assistant to complete a structured questionnaire. The structured questionnaire will ask questions about: your demographics, your current pregnancy and your latest CD4 count
2. You will then be assisted to complete a symptom screen and examined to complete the sign screen. The questions will be looking for symptoms of TB and the examination will be looking for signs of TB
3. If from the symptom and sign screen **YOU HAVE** any of the symptoms and signs of TB you will be administered Mantoux test and asked to come back to the clinic in 48 hours (2 days) to have your Mantoux test results read
4. If from the symptom and sign screen **YOU DO NOT HAVE** any of the symptoms and signs of TB you will be administered both Mantoux test tetanus toxoid antigen and asked to come back to us in 48 hours (2 days) to have your Mantoux test and tetanus toxoid antigen results read

5. After the first trimester of your pregnancy you will be asked to do Single view dose regulated shielded chest radiograph (unless otherwise indicated by the clinician offering care to you)
6. You will be asked to give us 3 samples of sputum if you have a productive cough
    a) *Sample 1-first spot sample on first contact with us (you will be given a sputum container to take home for 2nd sample)*
    b) *Sample 2- second sample will be collected early morning after 48 hours (when you will be coming back for the skin test readings)*
    c) *Sample 3- on return to clinic*

From the sputum samples we will do microscopy and culture

Risks of Taking Part in the Study

While on the study, the risks are perceived to be minimal, however the following may pose risk to you:

1. ***Loss of privacy****:* To protect your privacy research assistants who will be trained by the principal investigator on ethical issues before the start of the study and will maintain confidentiality. All paper records from interviews with us will be kept in locked cabinets and electronic records within the database will be password protected, and only data entry personnel, clinicians overseeing the database, and researchers involved on this

project will have access to them. In addition, patient names and identifiers will be removed from all data tables and records prior to data analysis.

2. ***The single view dose regulated shielded chest radiograph*** This should not be an issue because of the following reasons: the radiographs will be done after first trimester when the risk of damage to the unborn baby has been shown to be low, full size chest radiographs; which are recommended in pregnancy because of low dose skin entrance of radiation will be used in this study, mass miniature chest radiography with high dose skin entrance of radiation will not be used in this study, shielding to the fetus will be provided, a consultant radiologist will visit the study sites to ensure that the chest radiograph machines are the recommended ones for the study and to standardize the procedure, the radiographs will be done by experienced radiographer.

3. ***Mantoux Test and Tetanus Toxoid antigen***

Both these tests have been demonstrated to be safe in pregnancy. They rarely cause side effects. Local side effects on the site of injection include itchiness and swelling. Systemic side effects very rarely occur and include generalized rash and anaphylactic reaction. You are kindly requested to report any side effects to the research team in the clinic or give us a phone call on the numbers provided to you at the end of this consent form.

Benefits of Taking Part in the Study

There are no direct benefits to you for taking part in this study. While there is no direct benefit, if TB is diagnosed you will be referred for TB treatment and this will

ensure that we prevent transmission of TB to your unborn child and immediate family. In addition; we will know your TB status before we start you on anti-retrovirals that will help prevent transmission of HIV to your unborn child. The overall benefit of this study will be the expected improvement in patient care programs. This will result in benefits to the community of science as a whole, and may have a broader positive effect on health care for HIV/TB co-infected pregnant patients locally and the world at large.

Alternatives to Taking Part in the Study

You have no obligation to take part in this study. Instead of being in the study, you may continue with your usual care. If you are a patient now and decide not to participate, this will not affect your care in any way.

Confidentiality

Efforts will be made to maintain confidentiality. Your identity will be held in confidence in reports in which the study may be published. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the investigator and her research associates and the Moi University Institutional Research Ethics Committee.

Costs/Compensation

There will be no cost to you to participate in this study. You will be given 300 Kenyan Shillings has compensation for your transport cost. This will be for a one way return visit to the clinic to come back for the mantoux and tetanus toxoid test readings and for sputum collection. Half the amount of money will be given to you

on enrollment and the other half on return to the clinic for the skin test reading and sputum collection.

Questions about the Study

You can ask the study staff any questions that you may have about the study. They will be happy to answer any questions at any time during the study. If you have any questions regarding this study or your participation in it or you develop any problems because of your participation in this study, you may contact the clinic or the Principal Investigator using the following numbers:

Busia Clinic: 0727 478 613

Eldoret Clinic: 0727 870 882

Principal Investigator: 0722 273 443

Voluntary Nature of Study

Taking part in this study is voluntary. You may choose not to take part or may leave the study at any time. You are free to end the conversation at any time during the interview. Leaving the study will not result in any penalty or loss of benefits to which you are entitled.

Witness of Subject's Consent

The study has been explained to the subject. The subject has verbally expressed their willingness to participate in the study.

Witness 1: Signature…… Date ……..

Study Participant: Signature ……Date……

Appendix VII: Kiswahili Informed Consent (Ridhaa iliyotolewa kwa ufahamu)

Kauli ya kukubali

Utafiti huu unanuia kuchunguza kiwango cha kuenea kwa ugonjwa wa kifua kikuu miongoni mwa wanawake wajawazito wanaougua ugonjwa wa Ukimwi katika sehemu za magharibi mwa Kenya, na dhima ya uchunguzi wa kikliniki, kimaabara na kieksirei katika utambuzi wa kuwepo kwa ugonjwa huu katika wanawake hawa.

Azma ya Utafiti

Umealikwa kushiriki katika utafiti unaofanywa na watafiti kutoka Chuo Kikuu cha Nairobi (bewa la Tiba) ili kuchunguza dhima ya uchunguzi wa dalili na ishara katika utambuzi wa kifua kikuu katika wanawake wajawazito wanaougua ugonjwa wa Ukimwi katika shirika la AMPATH. Ugonjwa wa kifua kikuu ndio unaojulikana kuwaathiri wagonjwa wa Ukimwi sana. Utambuzi wa ugonjwa huu katika wanawake wajawazito unaweza kukawishwa na kutokuwepo kwa dalili maalum za mapema. Kuwepo kwa uchovu na unyonge wakati wa mimba ni dalili ambazo zinafanana na dalili za kwanza za kifua kikuu. Ugonjwa wa kifua kikuu ambao hujatibiwa unaweza ukahatarisha maisha yako au ya mtoto wako.

Aidha, kama sehemu ya kujitunza, utapewa madawa ya anti-retroviral ili kumkinga mtoto unayembeba kutokana na kuambukizwa ugonjwa wa Ukimwi. Ni muhimu utambuzi wa ugonjwa wa kifua kikuu, iwapo ugonjwa huu upo, ufanywe kabla ya kuanza kuyatumia madawa hayo. Sababu hii imewafanya wachunguzi

kutaka kujua njia iliyobora zaidi ya kutumia ili kutambua iwapo mwanamke anayeugua Ukimwi ana ugonjwa wa kifua kikuu.

Idadi ya washiriki katika utafiti

Iwapo utakubali kushiriki, utakuwa mmoja kati ya wanawake 183 wanaoshiriki. Wanawake hawa ni wajawazito na wanahudumiwa na kliniki za AMPATH zilizopo Eldoret na Busia.

Utaratibu wa utafiti

Iwapo utakubali kushiriki katika utafiti huu, basi utaombwa kuyafanya yafuatayo:

1. Utasaidiwa na naibu mtafiti aliyehitimu ili uweze kujaza hojaji maalum. Hojaji hii itakuwa na maswali kuhusu: demografia, mimba unayoibeba, na hesabu yako ya mwisho iliyofanywa ya idadi ya kinga mwilini.
2. Baadaye utaulizwa maswali kadhaa ili kujaribu kutambua ishara na dalili zinazohitajika. Maswali utakayoulizwa yatasaidia kuzitambua dalili za kifua kikuu na uchunguzi utakaofanyiwa pia utasaidia kuzitambua ishara za ugonjwa huu.
3. Iwapo matokeo ya uchunguzi huu wa dalili na ishara yataonyeshana kwamba UNA dalili na ishara zozote za ugonjwa wa kifua kikuu, utapewa chanjo maalum kisha utaagizwa kurudi baada ya masaa 48 (siku 2) ili matokeo ya chanjo hiyo yasomwe.
4. Iwapo matokeo ya uchunguzi wa dalili na ishara yataonyesha kuwa HUNA dalili na ishara zozote za ugonjwa wa kufua kikuu, basi utapewa chanjo maalum (Mantoux test) na ile ya pepopunda. Kisha utaagizwa kurudi

kwenye kliniki baada ya masaa 48 (siku 2) ili matokeo ya chanjo hizo yasomwe.

5. baada ya wiki kumi na tatu (13) za kwanza za mimba, utapigwa picha ya eksirei ya kifua (Iwapo hakutakuwa na maagizo ya kupinga kutoka kwa daktari au mhudumu wako)
6. Kisha utaombwa ulete sampuli tatu za kikohozi iwapo unakohoa.
    a. Sampuli 1 – sampuli ya kwanza itachukuliwa wakati huo *(utapewa kijichupa cha kuwekea kikohozi cha pili ubebe nyumbani ili uweke sampuli ya pili)*
    b. Sampuli 2– utaiweka katika kichupa hicho asubuhi na mapema baada ya masaa kumi na manne; 48 *(wakati utakapokuwa ukirudi kwenye kliniki kupima matokeo ya chanjo)*
    c. Sampuli 3 – itachukuliwa utakaporudi kwenye kliniki.

Sampuli hizo zitafanyiwa uchunguzi.

Hatari za kushiriki katika utafiti

Wakati tunafanya utafiti, hatari huonekana kuwa chache, hata hivyo, yafuatayo yanaweza kuwa hatari kwako:

1. ***Kutokuwa na usiri/faragha:*** ili kulinda usiri wako, wasaidizi wa utafiti watakuwa wamepewa mafunzo na mtafiti mkuu juu ya maswala ya maadili na watazingatia usiri. Pia rekodi zote za mahojiano kati yetu zitawekwa katika kabati zilizofungwa na rekodi zozote zitakazokuwa katika tarakilishi/kompyuta zitawekewa kizuizi (neno la siri) litakalokuwa linafahamika na anayehusika na uingizaji wa data, wale wanaofanya kazi

katika eneo hilo la database na watafiti wanaohusika katika utafiti huu. Zaidi ya hayo, majina ya wagonjwa na vitambulishi vyao vitatolewa kutoka kwa rekodi za data kabla ya kufanya uchanganuzi wa data.

2***. Eksirei ya kifua:*** upigaji wa picha hizi haufai kuwa tatizo kwa sababu zifuatazo: picha zitapigwa baada ya wiki 13 za kwanza za mimba wakati ambapo hatari zozote zinazoweza kumwathiri mtoto zimethibitishwa kuwa chache. Eksirei inayopendelewa ni ile inayorekebishwa ili kuhakikisha kwamba miale ya eksirei haipenyi sana ndani ya ngozi. Mtoto atakingwa kutokana na miale hii. Mshauri aliye na ujuzi wa kufanya eksirei atahusishwa ili kuhakikisha kwamba mashine za kufanyia eksirei ni zile zinazotakikana. Pia, mshauri huyu atasaidia kuhakikisha kwamba upigaji wa picha hizi umefanywa kwa njia inayofaa. Picha hizi zitapigwa na wapigaji eksirei walio na ujuzi.

3. ***Chanjo maalu (ya kubaini iwapo unaugua ugonjwa wa kifua kikuu na ya pepopunda:*** tayari imethibitishwa kwamba majaribio haya mawili hadhuru mimba kwa njia yoyote ile. Aidha, hayana athari zozote. Athari ambazo zaweza kujitokeza sana ni kujikuna na kuvimba katika sehemu uliyodungwa. Athari zingine ambazo hazijitokezi kwa urahisi ni kutokwa na vipele mwilini na athari mbaya kama vile kuvimba mwili. Unaombwa uripoti athari zozote zitakazojitokeza kwa watafiti katika kliniki au upige simu kwa namba ambazo umepewa mwishoni mwa fomu hii.

Manufaa/Faida za kushiriki katika utafiti huu

Kwanza kabisa, hakuna manufaa ya moja kwa moja ya kushiriki katika utafiti huu.

Hata hivyo, ikitambuliwa kwamba unagua ugonjwa wa kifua kikuu, basi utatibiwa ipasavyo jambo ambalo litazuia kumwambukiza mtoto wako na familia yako ugonjwa huu. Pia tutaweza kujua iwapo unaugua ugonjwa wa kifua kikuu kabla hatujaanza kukupa dawa za **anti-retroviral**. Kufanya hivi kutasaidia kumkinga mtoto wako kutokana na kuambukizwa ugonjwa wa Ukimwi. Faida ya kijumla ya utafiti huu ni kuwa itawezesha mgonjwa kushughulikiwa kwa njia bora zaidi jambo ambalo litaifaidi taaluma ya sayansi kwa jumla. Pia, matokeo ya utafiti huu yatakuwa na athari chanya (positive effect) kwa jumla katika kuwashughulikia wanawake wanaugua Ukimwi na kifua kikuu kwa wakati mmoja hapa nchini na duniani kwa jumla.

Uchaguzi wa kutoshiriki katika utafiti huu

Hulazimishwi kushiriki katika utafiti huu. Badala ya kushiriki katika utafiti huu, unaweza kuchagua kuendelea kupokea matibabu yako kama kawaida jambo ambalo halitaathiri huduma unayoipokea kwa njia yoyote ile.

Usiri

Tutatia jitihada katika kuhakikisha kwamba tumehifadhi usiri. Tutahakikisha kuwa hatujakutambulisha katika ripoti yoyote itakayochapishwa. Mashirika yoyote yatakayofanya ukaguzi na/au kuandika rekodi zako kwa sababu za kuthibitisha ubora na kuchanganua data ni kama vile mtafiti na wanashirikiana naye katika kuufanya utafiti na kamati ya maadili ya utafiti ya taasisi ya Moi.

Malipo/fidia: Hutahitajika kulipa chochote ili ushuruki katika utafiti huu. Utapewa shilingi 300 kugaramia nauli yako utakapokuwa ukirudi katika kliniki ili kufanyiwa uchunguzi zaidi.

Maswali kuhusu utafiti: Una uhuru wa kuwauliza watafiti maswali yoyote ambayo unawezakuwa nayo kuhusu utafiti huu. Watafurahia kuyajibu maswali yoyote, wakati wowote katika kipindi cha utafiti. Iwapo utakuwa na swali lolote kuhusu utafiti huu au ushiriki wako katika utafiti huu, au iwapo kutatokea matatizo yoyote yanayohusiana na kushiriki kwako katika utafiti huu, unaweza kuwasiliana na kliniki au mtafiti mkuu kupitia nambari za simu zifuatazo:

Kliniki ya Busia: 0727-478613

Kliniki ya Eldoret: 0727-870882

Mtafiti mkuu: 0722-273443

Uhiari unaohusishwa na utafiti huu

Kushiriki katika utafiti huu ni kwa hiari. Unaweza kuchagua kutoshiriki au kujiondoa katika utafiti huu wakati wowote. Una uhuru wa kusitisha mahojiano wakati wowote unaotaka. Kuamua kujitoa katika utafiti hakutaleta adhabu yoyote au kutopotea faida yoyote unayostahili kupata.

Kwa mashahidi

Mhusika ameelezewa mambo yote yanayohusiana na utafiti. Aidha, yeye amekiri kwa maneno yake mwenyewe kujitolea kwake kama mshiriki katika utafiti huu.

Mshahidi 1: Sahihi……Tarehe .....

Mshahidi 2: Sahihi……Tarehe.....

Appendix VII: Ethical Approval