ADDIS ABABA UNIVERSITY, COLLEGE OF HEALTH SCIENCE, SCHOOL OF GRADUATE STUDIES, DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY

Prevalence of Endometrial Tuberculosis among Patients Undergoing Endometrial Biopsy at Tikur Anbesa Specialized Hospital, Addis Ababa, Ethiopia

By: Sileshi Abdissa

April, 2013

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Table of Content Page

List of Figures

Page

List of Tables page

List of Annexes

Page

List of Abbreviations

AFB	Acid Fast Bacilli
AHRI	Armauer Hansen Research Institute
AIDS	Acquired Immunodeficiency Syndrome
ALIBP	Aklilu Lemma Institute of Pathobiology
BCG	Bacille Calmette Guerin
CTAB	Cetyl trimethyl ammonium bromide
DNA	Deoxyribonucleic Acid
dNTP	deoxynucleotide triph-osphate
DOT	Directly observed treatment
DR	Direct Repeat
DST	Drug susceptibility testing
EDTA	Ethyline diamine tetraacetic acid
EPTB	Extrapulmonary tuberculosis
FGTB	Female Genital Tuberculosis
GTB	Genital Tuberculosis
HIV	Human Immunodeficiency Virus
HPE	Histopathological Examination
HRZE	Isoniazid, Rifampicin, Pyrazinamide and Ethambutol
IGRA	Interferon Gamma Release Assay
ISs	Insertion sequences
LJ	Löwenstein-Jensen
MAC	Mycobacterium Avium Complex
MDR-TB	Multi-drug resistant TB
MOR	Minor operation room

MTC	*Mycobacterium Tuberculosis* complex
MVA	Manual Vacuum Aspirate
PCR	Polymerase Chain Reaction
PPD	Purified protein derivative
PTB	Pulmonary Tuberculosis
PZA	Pyrazinamide
QFT-G-IT	QuantiFERON TB-GOLD In-Tube
RD	Region of Difference
RFLP	Restriction fragment length polymorphism
rRNA	ribosomal Ribonucleic Acid
RvD	H37Rv Related Deletion
SDS	Sodium dedoceyl sulphate
SNPs	Single-nucleotide polymorphisms
SPSS	Statistical Packaging for Social Science
TAE	Tris-Acetic acid EDTA
TB	Tuberculosis
TBD1	Mycobacterium tuberculosis specific deletion
TDR-TB	Totally drug resistant TB
UK	United Kingdom
WHO	World Health Organization
XDR-TB	Extensively-drug resistant TB
ZN	Ziehl-Neelsen

Abstract

Background: Female genital tuberculosis (FGTB) is known to cause severe tubal disease leading to infertility and its incidence parallels closely with the overall prevalence of tuberculosis (TB) in a community. Its magnitude is underreported because diagnosis is difficult and requires invasive techniques. Investigation with advanced microbiological techniques may allow for easier, fast and correct diagnosis and treatment that may help to prevent complications.

Objective: The aim was to determine the prevalence of endometrial tuberculosis among women who underwent endometrial biopsy for evaluation of various conditions at a referral hospital and characterize the isolates.

Materials and Methods: A cross-sectional study was conducted on women for whom endometrial biopsy was done for the diagnosis and treatment purpose of different gynecologic problems at Tikur Anbessa Specialized Hospital (TASH), Gynecology outpatient Department (OPD), up on informed consent. During the data collection period, December 2011 to August 2012, a total of 152 participants were included in the study. The leftover biopsy samples were processed for culture on Löwenstein-Jensen media and subjected to DNA extraction and direct PCR using IS1081 primer pair. The culture isolates were further analyzed using deletion typing for species identification and multiplex PCR for genus typing.

Result: IS1081-PCR identified 7/152 (4.6%) biopsies as endometrial tuberculosis. Only four of the seven (4/152, or 2.6%) were positive by culture. The prevalence of endometrial Tuberculosis (TB) was thus 2.6% (4/152) but 4.6% (7/152) with IS1081-PCR. However, histological examination identified only 2/152 (1.3%) as suggestive of endometrial tuberculosis. Only one of these samples was positive with both IS1081-PCR and culture. All of the four isolates were *M. tuberculosis*. The agreement between the clinical diagnosis and IS1081-PCR and/or culture, was found to be 0.28. Taking culture as gold standard, the sensitivity and specificity of IS1081-PCR and histology were 100% and 98%, and 25% and 50% respectively.

Conclusion: We have shown that *M. tuberculosis* is relatively frequently encountered in endometrial biopsy but the true magnitude of endometrial TB requires thorough investigation and may be missed with histopathological examination alone.

Key Words; Endometrial TB, Genital TB, Histopathology, IS1081-PCR, *Mycobacterial* culture, *Mycobacterium* tuberculosis

Chapter One

1. Introduction

1.1. Background

Tuberculosis, a disease of broad host range, caused mainly by seven different species with common name of *Mycobacterium tuberculosis* complex (MTC), remain public health important despite the presence of modern therapeutics and vaccine. In 2011 a total of 8.7 million (13% co-infected with HIV) new cases reported globally. The deaths from TB were 1.4 million (990, 000 deaths among HIV-negative individuals and 430, 000 among people who were HIV-positive). Africa and Asia, geographically, are the highest TB burden continents. Asia attributes about 59% (India and China combined have almost 40%) of the world's TB cases. Approximately one quarter (26%) of the world's cases is from Arica region. Ethiopia, being among the high TB burden countries, was ranked 11th in the 2011 report. The global estimate of 2002, 141 cases per 100, 000, fell to an incidence rate of 128 cases per 100,000 population in 2010 (WHO, 2012).

Tuberculosis predominately presents with pulmonary disease, although extra-pulmonary TB (EPTB) is not uncommon (Tripathy and Tripathy, 1998). Genitourinary TB, comprising about 30%, is the second most common form of extra pulmonary TB (Weinberg and Boyd, 1988). The prevalence of FGTB increases in countries with high

Pulmonary Tuberculosis (PTB) burden. Five to 13% of all pulmonary patients develop GTB (Madhu and Davinder, 2000).

The low sensitivity and specificity of routine diagnostic methods and the paucity of the organism in clinical sample (biopsy) are the main factors for the less detection in the diagnosis of FGTB, in addition to the subtle presentation of the disease (Bhanu *et al.*, 2005). Therefore, Including advanced techniques as part of diagnosis is crucial in defining the magnitude of the problem and alerting early investigation, before serious complications occurred.

1.2. Literature Review

1.2.1. Mycobacterium Tuberculosis Complex

DNA-DNA hybridization studies of single species of *M. tuberculosis* and six other similar *Mycobacteria* species form a complex: *Mycobacterium tuberculosis* complex (MTC) (Brosch *et al.*, 2002). Despite their diversity in terms of their host tropism, metabolism, growth, environmental niche, epidemiology and pathogenicity, the group has 99.9% similarity at nucleotide level and identical 16s RNA sequence (Huard *et al.*, 2003, Boddinghaus *et al.*, 1990). The genotypic grouping of MTC, after sequencing, revealed that only two loci; KatG codon 463 CTG (Leu) and gyrA codon 95A ACC (Thr) were present at high frequency. Accordingly, based on the combination of polymorphism located at these sites; all isolates of *M. africanum*, *M. bovis* and *M.canetti* had the

characteristics of group 1 (KatG463 CTG (Leu) and gyrA95 ACC (Thr)). Whereas, *M. tuberculosis*, in addition to group1, fell in to group 2 and 3; KatG 463 CGG (Arg) and gyrA95 ACC (Thr), and KatG463 CGG (Arg) and gyrA95 AGC (Ser) respectively (Sreevatsan *et al.*, 1997). The member of MTC includes; the primary causative agent of TB, *M. tuberculosis;* the main causative agent of TB in west Africa, *M africanum* and *M. bovis*, including the vaccine strain *M.bovis BCG*, which is responsible for bovine TB (Kallenius *et al.*, 1999). The others, uncommon in human, are; *M. microti,* a pathogen of voles and rarely infecting humans (Rastogi *et al.*, 2001); *M. canettii*, a rare MTC strain that produces smooth and glossy colonies with all known cases so far isolated from individuals having been to the horn of Africa, (Van Soolingen and de Haas, 1997); *M. pinippedii*, also known as the seal bacillus (Cousins *et al.*, 2003); and *M. caprae*, primarily isolated from goats(Aranaz *et al.*, 1999).

1.2.1.1. Mycobacterium tuberculosis

Based on the relative broad host range of *M. bovis*, there was speculation that *M. tuberculosis* had evolved from *M. bovis*. However, the assumption was no more worthy after the whole genome of *M. tuberculosis* strain (H37Rv) and *M. bovis* strain (BCG) were sequenced and presence or absence of Direct repeats (DRs), H37Rv related deletions (RvD) and specific deletion 1 (tbD1) in species of MTC were identified (Cole *et al.*, 1998, Fleischmann *et al.*, 2002). The analysis of complete genome of *M. bovis* confirmed that there were no gene clusters that are confined exactly to *M. bovis* (Pan *et*

al., 2011). Presence or absence of *M. tuberculosis* specific deletion (tbD1) is the base for the division of *M.tuberculosis* strains into "modern" and ancestral strain, the former including representative of the major epidemics as Beijing, Haarlem and West Africa 2 *M. tuberculosis* cluster (Brosch *et al*., 2002). Moreover, DNA successive loss, like RD9 and other following deletion was recognized for an evolutionary lineage denoted by *M. africanum*, *M. microti,* and *M. bovis* (figure 1.1) that separated from the progenitor of the present *M. thuberculosis* strain prior to the occurrence of TbD1 (Brosch *et al*., 2002, Sreevatsan *et al*., 1997).

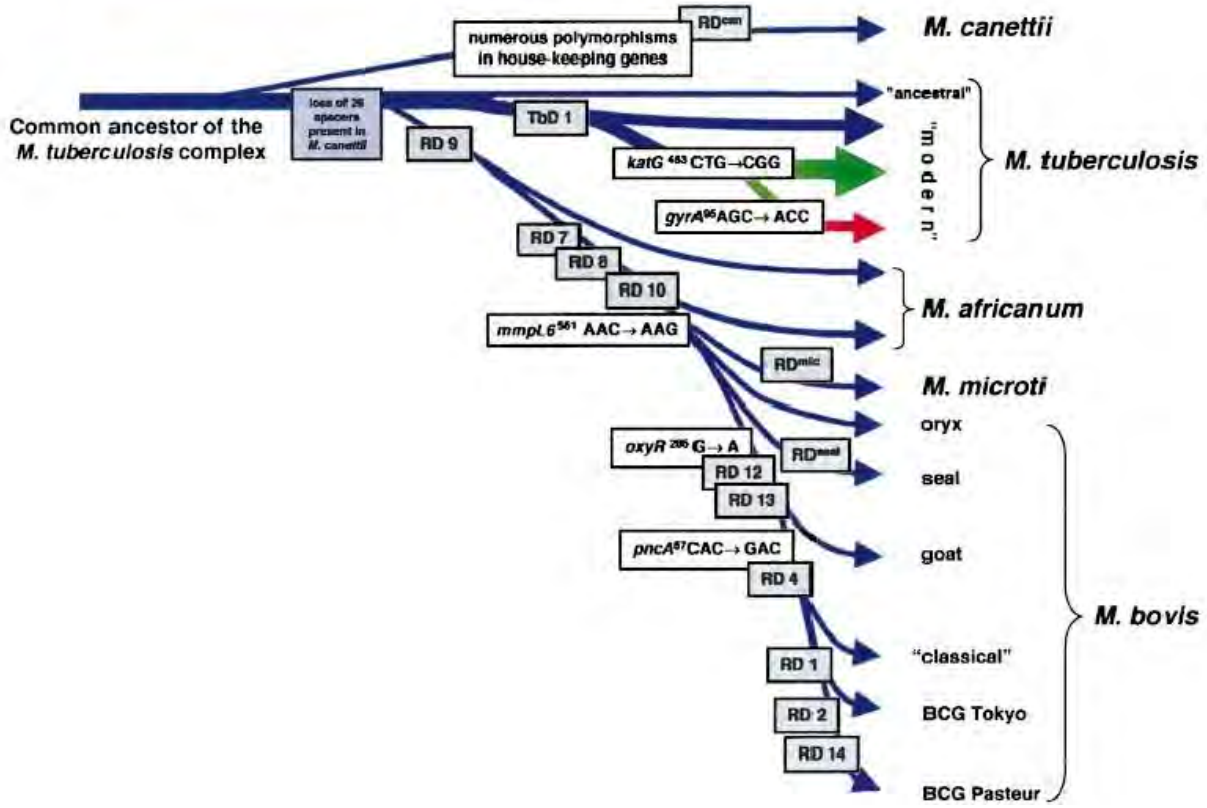

Figure1.2.1.1. Evolutionary Pathway of Tubercle Bacillii, illustrating successive loss of DNA in certain lineages (gray boxes), as proposed by Sreevatsan *et al.*, 1997 and sketched by Brosch *et al*, 2002. Blue arrows indicate that strains are characterized by katG463.CTG (Leu), gyrA95ACC (Thr), typical for group 1 organisms. Green arrows indicate that strains belong to group 2 characterized by katG463CGG (Arg), gyrA95ACC (Thr).The red arrow indicates that strains belong to group 3, characterized by katG463 CGG (Arg), gyrA95 AGC (Ser)

1.2.2. Natural Course of Tuberculosis

Aerosolized droplets of bacteria released from lung disease patients are the source of infection.

The resident alveolar macrophages phagocytes the inhaled 1to5µm particles and the adaptive Cellular immune response involving large number of chemokines and cytokines follow on (Henderson *et al.*, 1997, Roach *et al.*, 2002). This immune response apparently limits the infection, in 90% of immune-competent individuals, to the local draining lymph nodes ("Ghon complex"), and the lung parenchyma (Dannenberg, 1989). The granuloma formation containing activated T cells and *M. tuberculosis*-infected macrophages is an indicative for protective immunity. However, disease symptoms and associated pathology (tissue necrosis and cavitation) in 10% immune-competent individuals is through continual bacillary replication and release of bacilli from macrophage (Turner *et al.*, 2003). It remains obscure to differentiate tuberculosis incident cases either pulmonary or extra-pulmonary, whether it is due to reactivation of past infection or a recent transmission event. The epidemiological studies of contact tracing revealed that patients in high incidence area have the probability to be infected by both recent transmission and reactivation while TB due to reactivation prevails in low incidence area (Vynnycky and Fine, 1997, Vynnycky *et al.*, 2001).

1.2.3. Epidemiology of Female Genital TB

Many literatures agreed as it is difficult to know the epidemiology of female genital TB due to its difficulty in diagnosis (present with a symptom complex, resembling other diseases), the paucity of the organism in the biopsy, the invasive sampling techniques and lack of good set up for sampling and lab work. Most of the time, it was reported during investigation for infertility and is common in countries with high PTB. Thus, researchers' interest was on infertile women attending a given health institution to estimate the epidemiology GTB.

Therefore, it is not easy to get published journal on FGTB in many countries. In India, relatively, many literatures on FGTB among infertile women had conducted so far. Study done in one Hospital showed that from the general gynecological cases of infertile women, 7.2% cases were with genital problem (Jindal, 2006). Another study in same country on women suffering from infertility and undergoing laparascopic and hysteroscopic examination by Nested PCR revealed that 32.18% were positive for endometrial TB (Baxi *et al.*, 2011). A retrospective study in Iran reported that 1.3% of FGTB from all TB cases (Jahromi *et al.*, 2001). Inferile women were diagnosed for GTB using culture in South Africa with prevalence of 6% (Margolis *et al.*, 1992). Though Ethiopia is among the high TB burden countries,we could find only one study done on infertile women who were suspected for GTB. The prevalence was high 48% (14/25) (Abebe *et al.*, 2004), diagnosed uing culture, PCR and zeihl-Nielson techniques.

1.2.4. Diagnosis and Identification of *Mycobacterium tuberculosis* complex

The challenge in the laboratory diagnosis of TB is partly due to its fastidiousness and slow growing nature of the bacilli. Though identification of species is difficult by growing in culture and on conventional plate methods, there are some methods used in differentiation of species like *M. tuberculosis* from *M. bovis* and non-tuberculous from tuberculous *mycobacteria*.

1.2.4.1. Mantoux test

A purified protein derivative (PPD) of mycobacterium is used to distinguish TB-infected patients from those who have never been infected, based on the size of reaction through intra-dermal injection. However, the test does not discriminate between patients with previous vaccination, silent infection or active infection. Its false positivity compromises the value of the test and incentivizes the search for sensitive and specific laboratory tests (Thomas *et al*., 2003, Lee and Holzman, 2002).

1.2.4.2. Interferon Gamma Release Assay (IGRA)

Quantiferon-TB assay a recently developed tuberculin PPD assay used to detect *M. tuberculosis* in humans with a low to high risk of infection. In this quantitative in vitro

diagnostic assay, the interferon-gamma production using a single-step enzyme-linked immunosorbent assayed after over-night incubation of plasma derived from undiluted PPD stimulated whole blood. The measurability of interferon via an enzyme colorimetric assay by antibody and its low false positivity make it preferable to tuberculin skin test (Mazurek *et al.*, 2001). The two T-cell-based IGRAs are commercially available and widely used for diagnostic and research purposes: QuantiFERON TB-GOLD In-Tube (QFT-G-IT) (Cellestis Ltd., Carnegie, Australia) which includes an additional *M. tuberculosis*-specific antigen TB7.7 and T-SPOT.TB (Oxford Immunotec, Abingdon, UK).

1.2.4.3. Microscopy

Acid-Fast Staining remains the initial step for evaluation of TB using direct microscopic examination of the acid-fast bacillus (AFB) in a smear. Because it is cheap and fairly rapid, it is the only diagnostic test for TB, particularly in developing countries (Truffot-Pernot *et al.*, 2006). Among the three types of staining procedures: Ziehl-Neelsen, fluorochrome, and Kinyoun, Ziehl-Neelsen (carbolfuchsin) stain is preferable for organisms recovered from culture for its enhanced visualization of the morphologic features of the organism and superior specificity for identification of *M tuberculosis*. The bright fluorescence of stained bacteria under UV microscopy increases the sensitivity of detection at relatively low microscopic power by fluorochrome dye rhodamine (or

rhodamine-auramine) staining and is useful for screening. Kinyoun stains the bacilli without heating (Swaminathan *et al.*, 2010, Sharma *et al.*, 2005).

1.2.4.4. Culture

Culture on Löwenstein-Jensen (LJ) medium is still the gold standards for the diagnosis of active TB although many new molecular diagnostic methods have been developed. For resource limited countries, culture and Zeihl-Neelsen staining are used to confirm TB in patients with a clinical presumption of active disease. Six weeks or longer on solid media and 7-21days with liquid culture media will take the organism to grow. It is also important to test drug susceptibility (Rieder *et al.*, 2007).

1.2.4.5. Biochemical test

The differentiation of MTC by Biochemical analyses includes colony morphology, niacin accumulation test, growth in the presence of thiophen-2-carboxylic acid hydrazide (TCH; 2µg/ml), nitrate reduction on modified Dubos broth, and growth characteristics on Lebek medium and on bromcresol purple medium (induction of a pH-dependent change of color from blue to yellow). Oxygen preference in *Mycobacterium* isolates on Lebek (a semisolid medium) can be described as Aerophilic (growth on the surface) and microaerophilic (growth below the surface) (Normung, 1986). Nitrate reduction and niacin accumulation are the characteristics of *M.tubeculosis* (Niemann *et al.*, 2000). *M. bovis* is intrinsically resistant to pyrazinamide (PZA), major criterion for differentiation.

However, susceptibility to PZA among isolates of *M. bovis* has been reported in some studies (Niemann *et al.*, 2000, Wayne *et al.*, 1991). Biochemical tests have now been replaced with molecular techniques for the identification and classification of MTC.

1.2.4.6. Region of Difference based analysis

Various biological and molecular *Mycobacterial* characteristics have been utilized to identify

MTC isolates but have limited applicability as MTC taxonomical tools. A series of classical tests based upon growth, phenotypic, and biochemical properties have been traditionally used to segregate members of the MTC (Niemann *et al.*, 2000). However, together these tests can be slow, cumbersome, imprecise, non-reproducible, and time-consuming, and they may not give an unambiguous result in every case and many not be performed by every laboratory. To complement the classical tests for determination of MTC species, well-defined MTC lineage and subspecies restricted single-nucleotide polymorphisms (SNPs) have been used to specify certain MTC groupings through sequence analysis and/or digestion of PCR products followed by restriction fragment length polymorphism (PCR-RFLP) analysis (Niemann *et al.*, 2000). More recently, however, comparative genomics studied employing several different genetic hybridization strategies revealed regions of difference (RD) representing the loss of genetic material in *M. bovis* BCG compared to *M. tuberculosis* H37Rv (Gordon *et al.*,

1999) and these have been used in other studies to delineate species of the MTC (Huard *et al*., 2006).

1.2.4.7. Genus typing-Multiplex PCR

The genus typing (multiplex PCR protocol) uses six different primers. Firstly, it targets a sequence region within the 16S rRNA gene specific for the *Mycobacterium* genus. The two primers MYCGEN-F and MYCGEN-R are designed to amplify a specific PCR product from genomic DNA of all known *Mycobacteria*. Secondly, the PCR mix also includes primers that are specific for a hyper variable region of the 16S rRNA gene of *M. intracellulare* (MYCINT-F) and *M. avium* (MYCAV-R), giving one additional PCR product if the DNA template is any of these two species. Thirdly, species from the *M. tuberculosis* complex can also be identified due to the two primers (TB-F, TB-R) that target the MPB70 gene, specific for MTC (Katoch, 2004).

1.2.4.8. Spoligotyping

Spoligotyping is the most commonly used PCR-based technique to differentiate sub-species of *M. tuberculosis* strains (Groenen *et al*., 1993). *MTC* strains contain different chromosomal region with multiple direct repeats (DRs) of 36-bp interspersed by 35 to 41 bp DNA sequences of unique spacer. Based on which the detection of the 43 interspersed spacer sequences (initially ascertained in laboratory strain H37Rv and *M. bovis* BCG

vaccine strain P3) in the genomic DR region of MTC (Kamerbeek *et al.*, 1997, van Embden *et al.*, 2000). This typing method relies on determination of binary result (the presence or absence) of spacers in the in vitro-amplified DNA by hybridizing with labeled PCR-amplified DR locus of the tested strain to multiple membranes spotted 43 synthetic spacer Oligonucleotide covalently bound to a filter (Kamerbeek *et al.*, 1997). Results can be detected by chemiluminescence, and interpreted by computerized database.

1.2.4.9. Insertion sequence

The development of genetic tools, facilitated by the genomic rearrangement of bacteria, is crucial to know the mechanisms of genetic diversity of chromosomes. Specific DNA and protein mostly involved in genomic rearrangement. The induction of Homologous recombination, which takes place between repeated DNA sequences, is abundantly by insertion sequences (ISs) (Reif and Saedler, 1975). IS (is element) is a transposable element, which contains no genetic information other than that, necessary for its transposition (Singleton and Sainsbury, 2006). Thierry *et al* isolated a repetitive an IS-like element, IS6110, from *Mycobacterium* library. It has similarity with IS3 family and is specific to members of *Mycobacterium tuberculosis* complex. The copy numbers of IS6110 varies from 1to 25 between species and strains of MTC (Thierry *et al.*, 1990). In their an attempt to differentiate *M. tuberculosis* from *M. bovis*, Collins and Stephens, cloned a DNA sequence from *M. bovis*, and identified a DNA sequences with an insertion element that greatly differs from the IS3 family, but resembling that of IS256 of

Staphylococcus aureus (Collins and Stephens, 1991). This insertion element, IS1081 (is 1,324 bp in size) with copy number of 5 to 6, is exclusively present in MTC species and can be used to differentiate *M. bovis* BCG from others (van Soolingen *et al.*, 1992).

1.2.4.10. Histopathological Examination

Microscopically, after sectioning and staining with eosin-hemathoxyline staining, the inflammation produced with TB infection is granulomatous, with epithelioid macrophages and Langhans giant cells along with lymphocytes, plasma cells, maybe a few poly morph nuclear cells (PMN's), fibroblasts with collagen, and characteristic caseous necrosis in the center are characteristic feature (Pulimood., *et al* 2008). Tuberculosis bacilli can also be seen by zeilh-Nielson staining of biobsy.

1.2.5. Treatment and Prevention

1.2.5.1. Treatment

Standard regimens for new TB patients presumed, or known, to have drug-susceptible TB for the Intensive phase treatment includes isoniazid, rifampicin, pyrazinamide and ethambutol (HRZE), and HR are recommended for the continuation phase for 4 months. Dosing frequency for new TB patients can be daily or three times per week with acceptable alternative provided that the patient is receiving directly observed therapy

(DOT) and is not living with HIV or living in an HIV-prevalent setting (WHO/HTM/TB, 2009).

The current challenge with the treatment and prevention of the deadly disease, tuberculosis is the development of multi-drug resistant TB (MDR-TB) and extensively-drug resistant TB (XDR-TB) (WHO, 2010). An Isolate that is resistant to at least the two main first-line TB drugs rifampicin (RIF) and isoniazid (INH) is said to be MDR-TB whereas an MDR isolate which is further resistant to fluoroquinolone (FQ) and at least one of the second-line injectable agents: amikacin (AMK), kanamycin (Mukantabana) or capreomycin (CAP) is XDR-TB (WHO, 2009). The first cases of totally drug resistant TB (TDR-TB), resistant to all first line and second line anti-tuberculosis drugs, were reported from India (Udwadia *et al.*, 2012). The detection of drug-resistant TB by drug susceptibility testing (DST) in liquid or on solid media is laborious and takes several weeks to months to complete. The development of rapid molecular methods, actually endorsed by WHO, targets on specific molecular mutations associated with resistance against individual drugs and are able to perform within one or two days, important for the timely identification of drug resistance TB (WHO and TB, 2010). Some commercially available methods like Xpert MTB/RIF and GenoType MTBDR, which are detecting based on lack of probe hybridization to wild type loci, indirectly indicating presence of mutations are currently in use (Hillemann *et al.*, 2007, Hillemann *et al.*, 2009).

1.2.5.2. Vaccine

When the hypothesis was set out by Drs. Calmette and Gue´rin, to test that oral administration of bovine tubercle bacillus could transmit PTB (Calmette and Gue´rin, 1909), they serially sub cultured *Mycobacterium bovis in* ox bile medium and isolated Bacillus Calmette–Gue´rin (BCG) for the first time (Calmette and Gue´rin, 1909, Liu *et al*., 2009). However, after passage of 39 to 200 times, the strain was neither able to cause disease nor kill experimental animals, and did not revert also to virulence (Calmette and Gue´rin, 1909). The genomic analysis showed some deletions of RDs unique to the BCG strains that attributed to non-virulence of the bacterium. The three regions of difference identified when Mahairas *et al*, using subtractive hybridization and compared the genomic sequences of *M. bovis* and BCG for the first time were RD1, RD2, and RD3 (Mahairas *et al*., 1996). Later on, 16 other additional deletions, present in the genome of other *Mycobacterium tuberculosis*, recognized. Of these 11 were unique to M. bovis and 5 were only deleted from BCG (Behr *et al*., 1999). The specific deletion to all sub-strains of BCG, the region of deference labeled RD1 (9,454 bp), is the currently in use for vaccine of tuberculosis Worldwide. It has been concluded that the hypotheses, BCG was generated from the primary attenuating mutation of RD1, was reasonable. The low protective efficacy of the vaccine, which ranges from 0 to 80%, thought to be due to the heterogeneity of BCG strain (Behr, 2002).

Over the past decade, researchers have made significant progress in TB vaccine development, and a dozen TB vaccine candidates are now being evaluated in clinical trials. TB vaccination strategies follow two different approaches: pre-exposure

vaccination in order to prevent disease in individuals that have so far not encountered *M. tuberculosis* and post-exposure vaccination that aims at inhibiting disease outbreak in individuals that are already infected (Brennan and Thole, 2012). Current vaccines under clinical trials are summarized in Table 1.2.3.2.

Table 1.2.3.2: Tuberculosis vaccine candidates in clinical trials (Brennan and Thole, 2012)

Products	Product description	Type of vaccine	Indication	Status
AERAS-422	Recombinant BCG expressing mutated PfoA and overexpressing antigens 85A, 85B, and Rv3407	Recombinant Live	prime	Phase I
AdAg85A	Replication deficient adenovirus 5 vector expressing Mtb antigen 85A	Viral Vectored	Prime, Boost	Phase I
HyVac 4/AERAS - 404, + IC31	Adjuvanted recombinant protein composed of a fusion of Mtb antigens 85B and TB10.4	Recombinant Protein	Prime, Boost	Phase I
Hybrid - I+CAF01	Adjuvanted recombinant protein composed of Mtb antigens 85B and ESAT- 6	Recombinant Protein	Prime, Boost	Phase I
Hybrid 56 + IC31	Adjuvanted recombinant protein composed of Mtb antigens 85B, ESAT-6 and Rv2660	Recombinant Protein	Prime, Boost	Phase I
VPM 1002	rBCG Prague strain expressing listeriolysin and carries a urease deletion mutation	Recombinant Live	Prime	Phase II
M72 + AS01	Recombinant protein composed of a fusion of Mtb antigens Rv1196 and Rv0125 & adjuvant AS01	Recombinant Protein	Prime, Boost	Phase II
Hybrid - I+IC31	Adjuvanted recombinant protein composed of Mtb antigens 85B and ESAT- 6	Recombinant Protein	Prime, Boost	Phase II
RUTI	Fragmented Mtb cells	Whole cell, Inactivated or Disrupted	Immunotherapy	Phase II
AERAS - 402/Crucell	Ad35 Replication deficient adenovirus 35 vector expressing Mtb antigens 85A, 85B, TB10.4	Viral Vectored	Prime, Boost	Phase IIb
MVA85A/AERAS- 485	Modified vaccinia Ankara vector expressing Mtb antigen 85A	Viral Vectored	Prime, Boost	Phase IIb
Mw [M. indicus pranii (MIP)]	Whole cell saprophytic non - TB mycobacterium	Whole cell, Inactivated or Disrupted	Immunotherapy	Phase III

1.3. Statement of the Problem

Tuberculosis predominately presents with pulmonary disease, although EPTB is not uncommon (Jain, 2011). Genitourinary TB, comprising about 30%, is the second most common form of EPTB (Weinberg and Boyd, 1988). The prevalence of FGTB increases in countries with high PTB burden. Studies divulged that the most common and primary site of infection through hematogeneous route is fallopian tube (Parikh *et al.*, 1997). The spread of the organism from fallopian tube (Nogales-Ortiz F, 1979) involves infection of uterine endometrium (50%), ovaries (10-30%), cervix (3%), and vagina and vulva (<1%). The most frequent symptom FGTB is infertility (Parikh *et al.*, 1997), the consequence of irreversible damage to the fallopian tube. Five to 20% of all infertility cases are the result of salpingitis and endometritis, due to tuberculosis (Jahromi *et al.*, 2001, Saracoglu *et al.*, 1992), and even higher (39%-41%) among patients with tubal factor infertility. Infertility due to Tuberculosis may be caused diversely. Minimal damage to the tubes will result in ectopic pregnancy. Sixty percent of cases' tubal blockage is from severe damage to the tube. Adhesions of peritubal and masses on tuboovarian have been found in 47% of the cases (deVynck and Kruger, 1990). The prospective productivity in patients with genital TB (GTB) has been graded as poor varying from 16–38.2% (deVynck and Kruger, 1990, Parikh *et al.*, 1997). Although most cases of FGTB are asymptomatic, chronic pelvic inflammatory disease, menstrual irregularities, low grade fever, loss of weight and appetite, and Tubo-ovarian (T.O.) masses are also common manifestations of GTB (Varma, 1991, Sutherland, 1983). Depending upon the damage to the uterine cavity

and the endometrium, uterine Tuberculosis can be described as a mild, moderate, or sever (deVynck and Kruger, 1990).

1.4. Significance of the study

Considering the above facts, it is possible to postulate that TB could be one of the causes of genital problems in female patients in Ethiopia. However, there is limited information on the epidemiology of FGTB in general and endometrial TB in particular, in Ethiopia. Since Ethiopia is among the high TB burden countries, it can be expected that FGTB prevalent. This was also revealed by study on infertile women with suspicion of GTB by which the study reported that almost half (48%) of the cases were ascertained (Abebe *et al*., 2004). This study tried to define the magnitude of the problem in volunteer females with endometrial conditions and characterize the species of *Mycobacterium* known to cause FGTB so that it alerts the concerned body to take further action. From the general findings, it is thought that the sensitivity and specificity of the routine diagnostic method of endometrial TB, Histopathological examination (HPE), is affected by the paucity of the organism in clinical sample and missing of the infected site during sampling process. PCR technology and culture techniques, in the present study, are probably more sensitive to detect few bacilli.

Tentative diagnosis of a given disease condition based on clinical signs and symptoms is a common practice by physicians in Ethiopia. However, early suspicion would not be possible for GTB in most of the cases, as patients would not be known as cases of GTB

until irreversible damage like infertility has already happened. Therefore, due to the asymptomatic nature of the disease and its varied clinical presentation, studying the concordance of the different laboratory tests with clinical diagnosis is very important.

1.5. Objectives

1.5.1 General Objective

- To determine the prevalence of Endometrial TB among patients undergoing biopsy for endometrial conditions

1.5.1 Specific Objective

- To estimate the prevalence of endometrial tuberculosis among patients undergoing biopsy for endometrial conditions
- To identify the species of *Mycobacterium* from isolates of endometrial biopsy cultures
- To evaluate the diagnostic accuracy of the different diagnostic methods used in the detection of Endometrial TB

Chapter Two

Materials and Methods

2.1. Study Area and Study Participants

The study was conducted at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia. Since the Hospital is relatively well equipped with human power and material and serves as referral hospital, a great multitude of people from any corner of the country visit the hospital with different medical cases. However, patients from Addis Ababa and nearby are more commonly served. Samples from different health institutions of the country are also transported to the Hospital for further diagnosis and confirmation.

All volunteer females with endometrium problem whose endometrial biopsy was ordered for routine diagnosis had been included. The inclusion criteria were exclusive volunteers, and patients whose age is eighteen and above.

Non-volunteers, unconscious and patients with age below eighteen years and those who had taken anti TB drugs in the last two years were not included.

2.2. Study Design

Cross-sectional study design with convenient sampling technique was used to estimate the prevalence of Endometrial Tuberculosis and to type the circulating species of MTC. During data collection period, Dec. 2011 to Aug. 2012, 152 participants were included.

2.3. Sampling Technique

After briefly describing of the purpose, advantage, and disadvantage of the study to the study participants, the consent of every individual was taken. The volunteers were interviewed using semi-structured questionnaire. The consent form and the questionnaire were translated into simple (non-medical), local languages, and read for those who were illiterate. The samples were taken by Gynecologists using Manual Vacuum Aspirate (MVA) set for routine Histopathological diagnosis at minor operation room (MOR) of Gynecology department. Fresh, leftover biopsy sample was added aseptically into sterile universal bottles in 5 ml of 0.85 per cent saline solution. It was transported as soon as possible in an icebox at a temperature of 4°C to laboratory and was processed accordingly. Histopathological examination was done by experience pathologists at Tikur Anbassa Hospital as part of the clinical care of the patient.

2.4. Laboratory Methods

2.4.1. Culturing and Identification of *Mycobacterium*

Specimen processing and culturing for isolation of mycobacterium was carried out at TB laboratory of Akililu Lemma Institute of Patho-Biology (ALIPB) and at Armauer Hansen Research Institute (AHRI). In the laboratory, the tissue sample was homogenized in a sterile porcelain mortar or tissue grinder using 0.5-1ml sterile saline after sectioning of each tissue specimen in to fine pieces with a sterile scalpel or scissor. The homogenate was then decontaminated (Kubica *et al*., 1964) using 4% NaOH for 15 min and centrifuged at 3000 rpm for another 15 min. Two drops of phenol red indicator was added to the sediment after the supernatant was discarded and 2N HCl was added to neutralize. Neutralization was deemed to be achieved when the color of the solution was changed from purple to yellow. Then the sediment was inoculated immediately onto culture medium.

Two sets of Löwenstein-Jensen slants; one supplemented with 0.4% Sodium pyruvate (L-J pyruvate) and the other with glycerol (standard L-J) were prepared. After appropriate labeling and inoculation of the culture with 0.2-0.4ml (2-4 drops) of the centrifuged sediment, it was incubated aerobically at 37°C. The tubes were put in slant position for 5-7 days and then in upright position. All cultures were examined 72 hours after inoculation to check that liquid has completely evaporated, to tighten caps in order to prevent drying out of media, and to detect contaminants. The incubation period lasts for at least eight weeks, with weekly observation for discernible growth.

Identification was based on morphology, color, rate of growth, and the acid-fastness (confirmed by Ziehl-Neelsen (ZN) staining) (Truffot-Pernot *et al.*, 2006). Thereafter, isolates from the positive cultures were preserved with freezing media while at the same time heat killed in water bath at 80°C for 1 hour. The frozen and heat killed isolates were stored at -20°C for further mycobacteriology and molecular typing analysis.

2.4.2. Region of deference based Deletion Typing

Heat killed isolates were investigated by PCR for the presence or absence of RD9 using specific primers (Brosch *et al.*, 2002). The PCR amplification mixture used for RD9 typing was as follows: the HotStarTaq Master Mix (Qiagen, United Kingdom) was used for PCR, with primers described below (Table 2.5.2). The reaction mixture contained 10 µl of HotStarTaq Master Mix, 0.3 µl x 3 of each primer (flankR, F and Int), 2 µl DNA template and 7 µl distilled water to a final volume of 20 µl. Known *M. tuberculosis* was used as positive control while Qiagen water was used as negative control. The mixture was heated in Programmed Thermal Controller (Eppendorf, Hamburg, Germany) using an initial hot start of 95°C for 10 minutes followed by 35 cycles of 95°C for 1 minute; 55°C for 1 minute; and 72°C for 1 minute; a final extension step of 72°C for 10 minutes to complete the cycle. PCR products were electrophoresed in 1.5% agarose gel in 1XTAE running buffer. Ethidium bromide at ratio of 1: 10, 100bp DNA ladder and blue 6X loading dye at a ratio of 1:5 were used in electrophoresis. The gel was visualized in Multi–image UV light cabinet (EPi Chemi II DarkRoom).

The result was interpreted as *M. tuberculosis* (RD9 present) when a band of 396bp was observed comparing to commercially available ladder, divided by 100bp.

Table 2.5.2: Oligonucleotide primers used for RD9 typing of Mycobacterium isolates and sizes of the expected PCR products (Qiagen, United Kingdom)

Locus	Primer name	Primer sequence	Present	Absent
RD9	RD9_FlankF	AACACGGTCACGTTGTCGTG	396	575
	RD9_FlankR	CAAACCAGCAGCTGTCGTTG		
	RD9_IntenalF	TTGCTTCCCCGGTTCGTCTG		

2.4.3. Genus typing-Multiplex PCR

Multiplex PCR was run if species was not diagnosed and identified by deletion typing. Five primers (Table 2.5.3), with concentration of 10µM were used. DNA amplifications was done in thermocycler with 20 µl reaction volumes consisting: 2 µl of genomic DNA as a template, 10 µl HotstarTaqMasterMix (MgCL2, dNTP, Taq polymerase and PCR buffer) (Qiagen, United Kingdom) for each sample, 0.3 µl internal primer per sample, 0.3 µl forward and reverse. The reaction mixture was then heated in Programmed Thermal Controller (Eppendorf, Hamburg, Germany) cycle using the following amplification program: 95°C for 10 minutes for enzyme activation; 95°C for 1 minute for denaturation; 65°C for 0.5 minute for annealing; 72°C for 2 minutes for extension; involving 35 cycles all in all; and final extension at 72°C for 10 minutes. The product was electrophoresed in 1.5% agarose gel in 1XTAE running buffer. Ethidium bromide at ratio of 1: 20,000, 100bp DNA ladder, and blue 6x loading dye, at a ratio of 1:5, was used in gel

electrophoresis. After running electrophoresis, the gel was visualized using Multi Image Light Cabinet (EPi Chemi II DarkRoom) and photograph was taken. All members of the *Mycobacteria* genus gave a PCR product of 1030bp with the primers MYCGEN-F/MYCGEN-R (Katoch, 2004). *M. avium*, or subspecies including *M. paratuberculosis*, generated a PCR fragment of 180bp (primers MYCGEN-F/MYCAV-R) in addition to the 1030 bp genus product. Isolates from the *M. tuberculosis* complex produced a PCR fragment of 372bp (primers TB1-F/TB1-R) beside the 1030bp genus product. The quality control for this multiplex PCR was as follows: The positive controls for *M. avium*, *a*nd *M. tuberculosis* (H37Rv) always give two bands; the genus specific PCR product and the respective species-specific PCR product.

Table 2.5.3: Primers, primer sequence and interpretation of mycobacterium genus and mycobacterium complex (Qiagen, United Kingdom)

Primer Name	Primer Sequence	Present	Interpretation
MYCGEN-F	5'-AGA GTT TGA TCC TGG CTC AG-3'	1030bp	Genus *Mycobacterium*
MYCGEN-R	5'-TGC ACA CAG GCC ACA AGG GA-3'		
MYCAV-R	5'-ACC AGA AGA CAT GCG TCT TG-3'	180bp	*M.avium* Complex
TB1-F	5'-GAA CAA TCC GGA GTT GAC AA-3'	372bp	MTC
TB1-R	5'-AGC ACG CTG TCA ATC ATG TA-3'		

2.4.4. DNA Extraction

DNA from biopsy was purified as follows. The endometrial tissue was chopped finely using a sterile scalpel and homogenized manually in 1×TE buffer (TRIS – EDTA –10 mM Tris.Cl. *p*H 8.0; 1mM EDTA *p*H 8.0) until the solution became turbid, and

transferred to 1.5 ml of eppindrof tube. The supernatant was discarded after centrifuging at 1200 rpm for 20 minutes. By repeating pipetting, pellets were re-suspended in 500µl of TE buffer, and 50µl of 10 mg/ml lysozyme was added, mixed well and incubated for 1h at 37°C. Seventy micro-liter sodium dedocyl sulphate (Sigma, St.Louis, Mo.) with concentration of 10g/ml and 6µl of 10 mg/ml proteinase K (VWR international Ltd., poole, BH151TD, England) were then added, mixed, and incubated for 10 min at 65°C. Afterwards, 100 µl of 5 M NaCl was added and vortexed and following the addition of 80 µL of pre-warmed Cetyl trimethyl ammonium bromide (CTAB) /NaCl (Sigma, St. Louis, Mo.) in pure water, and the mixture was incubated at 65°C for 10 min. Approximately equal volume (700 - 800 µl) of readymade phenol:chloroform:isoamyl alcohol (VWR international Ltd., poole, BH151TD, England) in proportion of 25:24:1 was added, after vortexed for at least 10 seconds and Centrifuging for 10 min at 12,000 rpm. The resultant upper phase was transferred to a clean tube with 0.6 volume of isopropanol and mixed gently. The tubes were then moved slowly upside down to precipitate the nucleic acid, and would be incubated at -20 overnight. Spun in a Microfuge for 15 min at 12,000 rpm, the precipitate was washed by 70% cold ethanol and the supernatant was removed. The pellet was permitted to air dry for 15 minutes and above. Finally, it was re-suspended in 1XTris-EDTA (Sigma, St.Louis, Mo.) solution (from 20µ to 50µ) based on

the size of the pellet for PCR amplification. Positive control, known isolate from culture, and negative control, TE buffer, were used in the whole procedure.

IS1081-PCR

The amplification by the thermocyler by specific primers (Table 2.5.4) for the multiple copies of insertion sequence of IS1081 (Eurofins MWG Operon) included an initial denaturation at 94°C for 10 min followed by 35 cycles each of denaturation at 94°C for 1 min, annealing at 65°C for 0.5 min, and extension at 72°C for 2 min. The extension step in the 35th cycle was held for 10 min before the samples were shifted to 4°C for storage. By agarose gel electrophoresis system (BIO RAD, UK), the product was electrophoresed at 110v for 35 to 45 min in 1.5% agarose gel in 1XTAE running buffer. Ethidium Bromide at a ratio of 1:20,000, 100 bp DNA reference ladder and blue 6X loading dye in proportion of 1:5 were used in agarose gel electrophoresis. The gel was visualized using Multi Image Light Cabinet (EPi Chemi II DarkRoom) and photograph was taken. The result was interpreted as MTC when the band of 136bp was seen in reference to the 100bp ladder.

Table 2.5.4: Primer and primer sequence for PCR-IS1081 (Eurofins MWG Operon)

PCR	Primer	Sequence (5'-3')	amplicon size (bp)
IS1081	F_2	CTGCTCTCGACGTTAATCGCCG	136
	R_2	GGCACGGGTGTCGGAATCACG	

2.4.5. The overall procedure of the lab

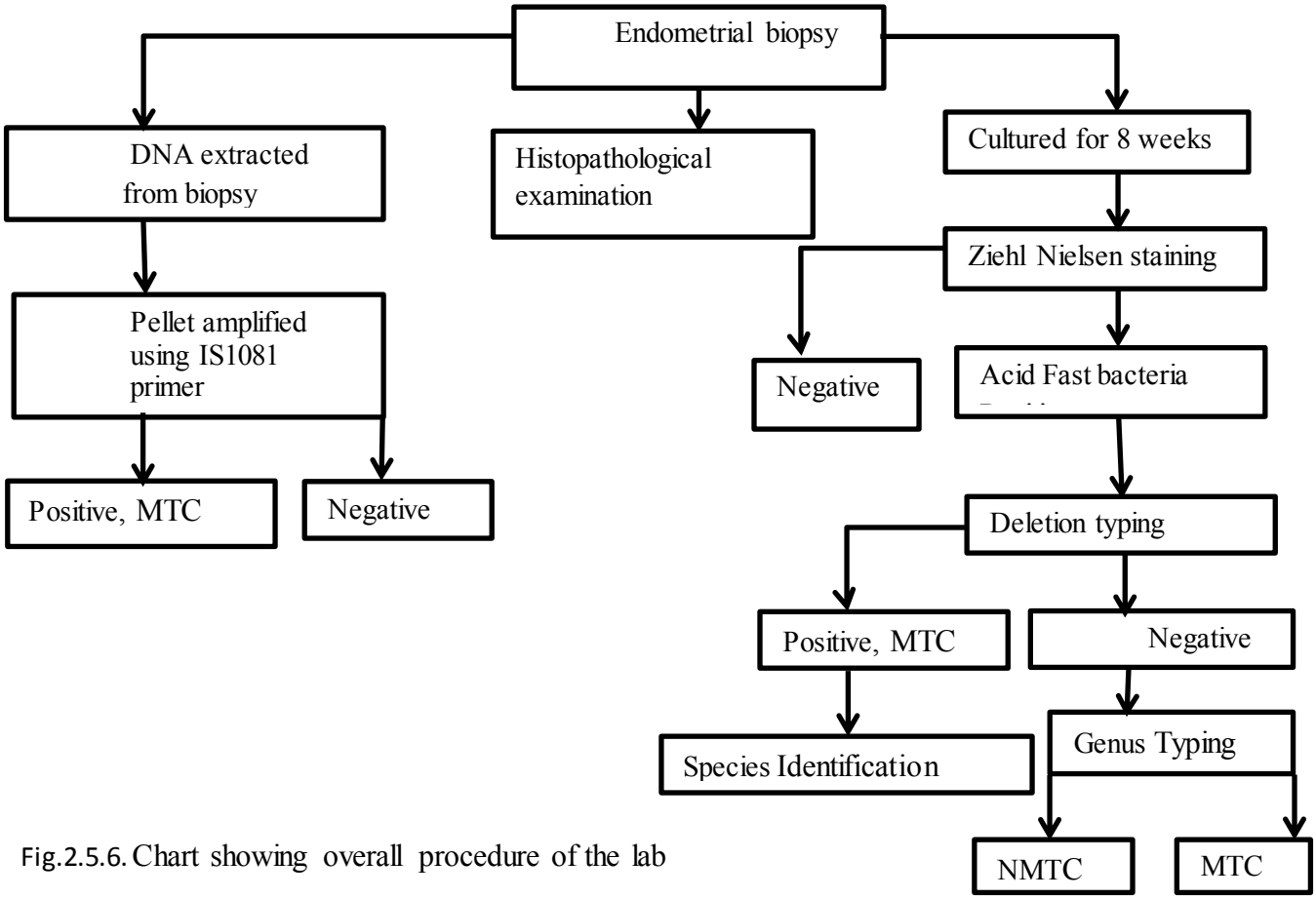

Fig.2.5.6. Chart showing overall procedure of the lab

2.5. Quality Assurance

The questionnaires were cross-checked for correct filling by the investigators. The functionality and the sterility of the equipment for transportation and material for sample taking were carefully monitored. Care was taken in preparing L.J medium, and then it was put at 37°C for 48 hours to check for contamination. Positive and negative controls were run together with all steps of DNA extraction and PCR amplification procedures. The area of PCR mix was cleaned with "DNA away" before and after each mix. We used a separate mix room and amplified the mix in a different room to prevent contamination.

2.6. Statistical Analysis

Data were entered and cleared using EpiData version 3.1. Then it was exported to SPSS software version 20 for analysis.

Descriptive statistics for age, marital status, region of the participant, and to determine the prevalence was of endometrial TB. The mean and standard deviation for age was also calculated. Crosstabs (kappa) was used for clinical diagnosis versus lab result, and for method evaluation respectively.

2.7. Ethical Consideration

The study was approved by Departmental Ethical Review and Research Committee (DERC) of department of Medical Microbiology, Immunology and Parasitology (DMIP), College of Health Science, Addis Ababa University, AHRI/ALERT Ethical Committee (AAEC), and departmental Ethical Committee of Gynecology and Obstetrics of Tikur Anbessa Specialized Hospital. After, clearly stating the purpose, merit and demerit of the study in local languages, written informed consent was obtained from all participants.

2.8. Result communication

All participants who were positive for endometrial TB were treated with first line anti TB drugs through their physician, except one patient who was not alive at the time.

Chapter Three

Result

3.1. Socio-demographic background of the Study participants

A total of 152 participants were included among patients with endometrial problem and whose endometrial biopsy was taken for routine histo-pathological investigation. The participants' age range were from 20-75 years with mean of 38.37 ± 10 years. Most of the participants were in the age category of 34-40 years relatively (Table 3.1). Majority of the patients were married (73%) and came from Addis Ababa (58.6%).

Table 3.1: Socio-demographic background of the study participants, December, 2011 to August, 2012, Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

	Range	Frequency	%
Age	20-26	16	10.5
	27-33	31	20.4
	34-40	54	33.5
	41-47	23	15.1
	48-54	19	12.5
	55-61	5	3.3
	62-68	2	1.3
	>/=69	2	1.3
Region	Addis Ababa	89	58.6
	Outside Addis Ababa	63	41.4
Marital status	Unmarried	29	19.1
	Married	111	73
	Divorced	8	5.3
	Widowed	4	2.6

3.2. Distribution Endometrial TB with Clinical Background of the Participant

Participants were interviewed for their related clinical background using questionnaire and their medical charts were also reviewed for further clinical data. As shown in Table 3.2, the major complaint of the study participants was menses irregularity (73.7%) followed by chronic pelvic pain (59.2%). Of the participants with complaints about chronic pelvic pain, 6.7% (6/90) had endometrial TB whereas, 7.7% (5/65) of the participants who had infertility problem were endometrial TB positive patients. Some of the infertile cases (29/65 or 44.6%) visited the Hospital for their infertility problem and 13.8% (4/29) of them were positive for endometrial TB. Three (2.7%) participants, with menses irregularities, were positive for endometrial TB. Only 71% of the participants were voluntarily screened for HIV and none of the HIV positive women had associated endometrial TB.

Table 3.2: Distribution of Endometrial TB with Clinical Background of the Participants, from December, 2011 to August, 2012, Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

Clinical background		**Frequency (Total 152)**	**Relative frequency (%)**	**Endometrial TB**		**Positive % (combined)**
				Culture positive	**IS1081-PCR only**	
Primary infertility		43	28.3	1	3	7.7
Secondary infertility		22	14.5	1	0	
Investigated for infertility problem		29	19.1	2	2	13.8
Menses disturbance		112	73.7	1	2	2.7
	Menorrhagia	67	59.8	1	0	
	Amenorrhea	24	21.4	0	1	
	Oligomenorrhea	21	18.8	0	1	
Chronic Pelvic pain		90	59.2	4	2	6.7
HIV screen result	Positive	9	5.9	0	0	0.0
	Negative	99	65.1	2	2	4.0
	Not tested	44	28.9	2	1	6.8
Anti TB before two years		13	8.5	0	0	0.0

3.3. Prevalence of Endometrial Tuberculosis

The prevalence of endometrial TB was determined by culture with species confirmed by RD and IS1081-PCR for MTC. Consequently, 7(4.6%) were found to be MTC by IS1081-PCR out of which only 4(2.6%) were culture positive and confirmed as *M. tuberculosis* by RD9. Only two samples were positive for endometrial TB by Histopathological examination. All the three diagnostic techniques identified 8(5.3%) participants as endometrial TB positive (Table 3. 3).

Table 3.3: Detection of M. tuberculosis with different diagnostic methods, from December, 2011 to August, 2012, Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

Assays	No. of samples	Positive result (%)
PCR-IS1081	152	**7(4.6)**
Culture with AFB confirmed	152	5(3.3)
Culture with species confirmed	152	**4(2.6)**
Histo-pathology	152	2(1.3)
Combination of Culture and IS1081-PCR	152	**7(4.6)**
All tests	152	8(5.3)

DNA extracted pellet from biopsy was amplified with IS1081 primer and produced band at 136 comparable with standard 100bp ladder and known positive control, Fig. 3.3.

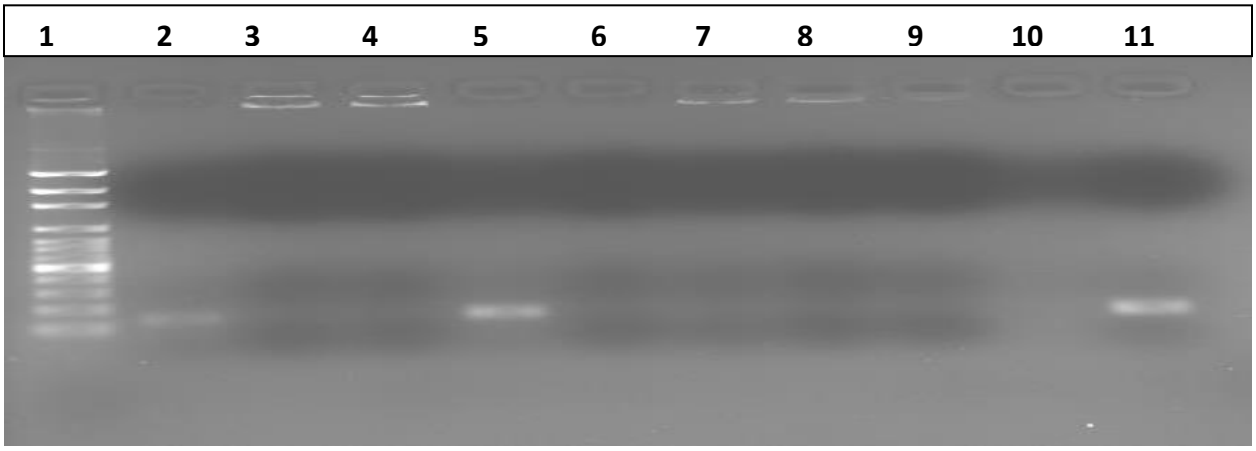

Figure 3.3: Selected slide showing gel picture of DNA amplified with IS1081primer. Lane 1= ladder (100bp), lane 2 & 5 positive for MTC*, lane 3, 4, 6, 7, 8 & 9 are negative for MTC, 10 = negative control and lane11 = positive control (H37Rv)

*MTC - *Mycobacterium Tuberculosis* Complex

3.4. Region of Difference Based Species Identification and Multiplex PCR Genus Typing.

After confirming the acid fastness of the isolates from the culture by Ziehl-Neelsen, further investigations conducted to differentiate the species among MTC by presence or absence of RD9. All AFB confirmed were *M. tuberculosis* (RD9 present) (Fig. 3.4.1), except one, which was later affirmed as not among the member of MTC or not genus of *Mycobacterium* by genus typing (Fig.3.4.2).

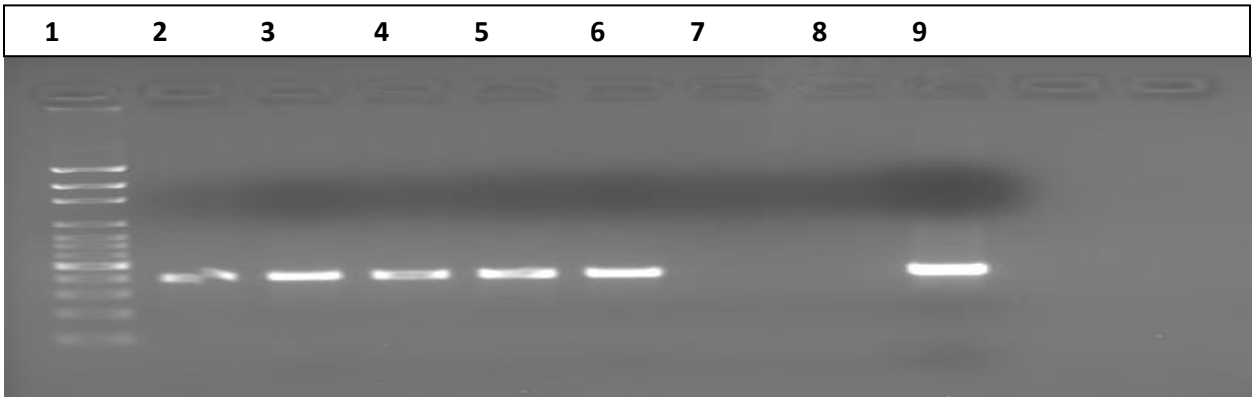

Figure 3.4.1: Gel picture showing Isolates of *Mycobacterium* characterized for species identification with RD9 Primer. Description; Lane 1 = ladder (100bp), lane 2 through 6 = M. tuberculosis (396bp), lane 7 = negative, lane 8 = negative control, lane 9 = positive control (H37Rv)

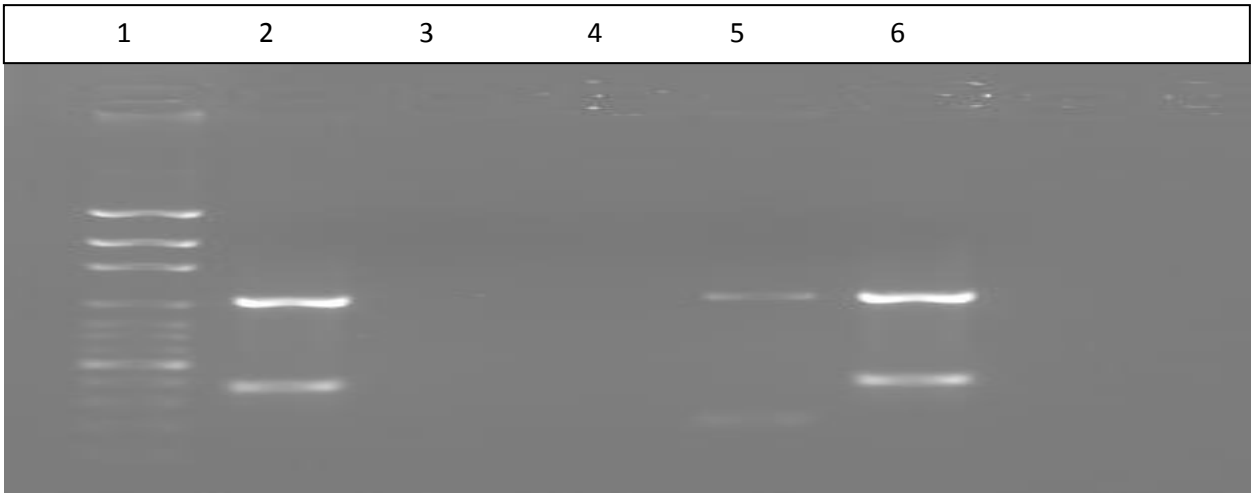

Figure 3.4.2: Gel picture showing Genus typing of *Mycobacterium* Isolates using Multiplex PCR. Lane 1 = ladder (100bp), lane 2= known M.tb, lane 3 = negative (was negative for RD9), lane 4 = negative control, lane 5 = positive control (MAV†, 180bp) and lane 6= positive control (M. tb, H37Rv, 372bp)

MAV† = *Mycobacterium avium* Complex

3.5. Agreement between Clinical criteria and Culture and/or IS1081 PCR

The distribution of endometrial TB among suspected and non-suspected cases were analyzed and found to be prevalent in patients that were not suggested by the physicians (3.4%, or 5/146) with measure of agreement (kappa) 0.28 (Table 3.5.1) with both culture and/or IS1081-PCR and 75% participants, who were culture positive only, were not suspected by physicians with kappa value of 0.17 (Table 3.5.2).

Table 3.5.1: Diagnosis by clinical criteria for endometrial TB versus the rate of positivity by culture, with species confirmed, and/or IS1081-PCR, from December, 2011 to August, 2012, Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

	Total (n=15)	Culture +IS1081-PCR		Sensitivity %	Specificity %	PPV	NPV	kappa
		Positive	Negative					
Clinically Suspected for endometrial TB?	yes	2	4	28.6	97.2	33.3	96.6	0.28
	no	5	141					

Table 3.5.2: Diagnosis by clinical criteria for endometrial TB versus the rate of positivity by culture only, December, 2011 to August, 2012, Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

	Total (n=152)	Culture		Sensitivity %	Specificity %	PPV %	NPV %	kappa
		Positive	Negative					
Clinically Suspected for TB?	yes	1	5	25.0	96.6	16.7	97.9	0.174
	no	3	143					

3.6. Evaluation of Histopathological Examination and IS1081-PCR with Culture

The performance of histopathological examination and IS1081-PCR were evaluated against culture. All culture positive samples were positive by IS1081-PCR, and one (50%) sample has concordant result between histopathology and culture (Table 3.6).

Table 3.6: Comparison of IS1081-PCR and histopathology with culture in diagnosing endometrial TB, from December, 2011 to August, 2012, Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

Assays	**Result** Total (n=152)	**culture positive with species confirmed**					**Kappa value**
		Positive	Negative	Sensitivity (%)	Specificity (%)	PPV (%)	
IS1081-PCR				100	98	57	0.718
	Positive	4	3				
	Negative	0	145				
Histology				25	50	50	0.321
	Positive	1	1				
	Negative	3	147				

Chapter Four

Discussion

In this study, we have described the prevalence of endometrial TB on volunteer patients who gave endometrial biopsy to Gynecology Outpatient Department (OPD) of Tikur Anbessa Specialized Hospital, Ethiopia, for histopathological examination of any suspected diseases from leftover biopsy. The prevalence of endometrial TB was 2.6% (4/152) with culture but 4.6% (7/152) with IS1081-PCR. As to our knowledge, there was no much literature on the prevalence of endometrial TB. One study has shown how much the disease was of public importance with prevalence of 48% (Abebe *et al*., 2004). However, the participants in the present study were by far different in that all were not infertile and not clinically suspected for GTB. In addition, the anatomical site of sample in our study was also only from endometrium rather than including other organs of the genital tract, which would thus underestimate the prevalence of the disease. A retrospective study in Iran (Jahromi *et al*., 2001) reported 1.3% of all TB cases were with GTB. The inconsistency of this report with our finding could be due to the difference in overall prevalence of TB in the two countries i.e. the prevalence of TB in the community is lower in Iran than in Ethiopia, the difference in diagnostic techniques and the difference in study participants as our study participants were women with genital problem. On the other hand, a similar study on general gynecological admissions identified 2.8% of GTB and 7.2% from infertile cases with gynecological problem (Jindal, 2006) with the help of a simplified TB algorithm. The inclusion of

Mycobacteriological tests and molecular techniques could be justification for the higher report in our study though India is among the high TB burden countries.

Most studies in Africa and Asian countries have given attention only to infertile women to estimate the contribution of GTB to infertility, to understand the related clinical signs and symptoms and the consequence of treatment on conceiving. A report from Tyerberg Hospital of South Africa (Margolis *et al*., 1992), a country with high TB burden than Ethiopia, showed an incidence of 6% culture positive GTB in an infertile population. Similar study conducted in India using Hysterosalpinography identified 6.3% GTB from all patients underwent through the procedure (Chavhan *et al*., 2004) which was in agreement with our study. However, the participants in the present study are less likely to be with GTB since not all were infertile. PCR, a sensitive technique, might increase the chance of detecting low number and dead bacilli.

Majority of the positive study participants (71.4%) were in the high reproductive age group (20-33). No patient in the menopause was positive to endometrial TB in this study. This is in agreement with a finding from India (Thangappah *et al*., 2011), reported 83.3% was less than 30 years, and 82% were in the age group of 21-30 (Kohli *et al*, 2011).

Infertility is the most commonly reported symptom in GTB (Parikh *et al*., 1997). In the current study, of the 65 (42.8%) women presented with infertility, 43 (66.2%) participants were incapable of conceiving an offspring in their life time and 22 participants had conceived one or more in the past and became infertile. This result was similar with the study conducted on 196 infertile women (Kulshrestha *et al*., 2011) in which 70.4% participants were cases of primary infertility. Another study also revealed

that 71% of the total 100 infertile women presented with primary infertility (Kohli *et al*, 2011). Almost all of the endometrial TB positive cases (6/7) were from patients who were complaining of chronic pelvic pain and the majority of them, 7.7% (5/65), were from the infertile cases, which is similar with the study in south Africa (Jindal, 2006) that reported 7.2% culture positive GTB from infertile cases. Of the 29 infertile patients, who visited the Hospital for their infertility problem, 4 (13.8%) were positive for endometrial TB. This is in line with the generally accepted fact that infertility is the number one symptom in GTB (Parikh *et al*., 1997).

Menstrual irregularity was the other commonly seen (73.7%) symptom in this study. The most common type being menorrhagia (59.8%) followed by amenorrhea (21.4%) and oligomenorrhea (18.8%). This was similar with a study from Uganda (Othieno *et al*., 2008) which reported that menorrhagia (35.9%) was the predominant symptom, but amenorrhea was least. Contrarily, oligomenorrhea was the most common complaint in the other study (Kohli *et al*., 2011).

Literature review revealed that *M. tuberculosis* is the most predominant among the MTC species in PTB and EPTB (Kallenius *et al*., 1999). Similarly, in the present study, all isolates were confirmed as *M. tuberculosis* by deletion typing, except for one isolate. We did genus typing and it was negative too. There are conditions in which other bacteria family of Actinomycetes (cornybacteria and Nocardia) known to cause pulmonary, cutaneous and sub-cutaneous disease, appeared as acid fast by Ziehl Neelson staining. Halpern *et al* found Rhodococcus equi, occurring as acid fast coccobacili, cause pneumonia (Halpern *et al*., 1994). The report from lymph node of cattle by Michael *et al*

showed acid-fast positive actinobacillus, known to infect animals and humans (Michael *et al.*, 2007b). Since the organism is ubiquitous in the environment, it could also be contaminant during sampling.

In the diagnosis of endometrial TB, a high degree of suspicion was aided; by active extra-genital TB, characteristics features on hystrosalpinography, evidence of calcification/ complex adnexal mass by scan, and contact history of the patients. As a result, six patients had been suspected for endometrial TB and two of them were positive for endometrial TB. One patient was positive by culture, IS1081-PCR and histology, but the other was detected only by IS1081-PCR. The overall kappa agreement between clinical criteria and laboratory examinations, culture and/or IS1081 and culture only was found to be 0.28 and 0.17 respectively. Endometrial TB was more prevalent in patients not suspected for endometrial TB (3.4%). This could be due to sub-clinical illness when women are still asymptomatic and early disease with low number of bacilli, and before structural damage to the tubes has taken place.

Rapid diagnosis and treatment of EPTB is decisive to lessen morbidity and mortality due to TB. Sensitive and specific methodology in the diagnosis of biopsy from genital organ for TB is crucial in managing the disease easily. Different researchers evaluated the performance of PCR to the conventional culture method in the diagnosis of TB from body fluid and biopsy. However, the difference in the primer used and nature of the sample determines its sensitivity and specificity. In our finding, all culture positive (4/4) were also positive by IS1081-PCR with sensitivity, specificity, PPV and NPV of 100%, 98%, 57% and 100% respectively. Other groups who evaluated the performance of

IS1081-PCR from lymph node (Michael *et al*., 2007a) and plural effusion (Bahador *et al*., 2005) showed that the sensitivity was 91% and 84.6% respectively. Positive agreement between culture and IS1081-PCR was observed in this study as shown by k=0.718 (good agreement). In contrary, Derese *et al* reported 23.4% sensitivity on comparison of IS1081-PCR with standard culture from lymph node (Derese *et al*., 2012). It is likely that the increased sensitivity of IS1081-PCR in our result reflects the small number of culture positive samples or good lyses of the *Mycobacterial* cell wall and dissociation of DNA from particulate matter in the crude homogenate that would allow the recovery of supernatant after centrifugation and enough sample proportion used for extraction. The three (2%) culture negative but IS1081-PCR positive samples should be cautiously seen about the discrepancy result as dead bacilli during storage or decontamination and absence or small number of bacteria in the sample portion of culture might contribute to the less growth detected by culture.

Histopathology examination is routinely used for diagnosis of endometrial TB. The method, providing characteristics feature of *M. tuberculosis* is easy, quick and cheap (Sathe *et al*., 1979). The primary aim of sample collection in this study was doing HPE using haematoxylin and eosin staining for routine diagnosis of cancer and other diseases. Accordingly, two (1.3%) samples were positive for tuberculosis endometritis. One sample has concordant result with both culture and IS1081-PCR, but the other was not positive by either of the two. The agreement between culture and histopathology was found to be 0.321. A study in India (Thangappah *et al*., 2011) revealed that histopathology was more sensitive (6.9 vs. 5.6), but was compared against clinical criteria that had diagnosed as probably GTB, using hystrosalpinography, laparascopy, ultrasound

and other hematological tests. The possible reasons for the less sensitivity of the histopathological examination in the present study could be sampling before occurrence of inflammation or granuloma formation on endometrium and the sampled site may not represent the infected area.

Chapter Five

Conclusion and Recommendations

In the high TB burden countries, like Ethiopia, the epidemiology of EPTB should also be known in parallel to PTB. GTB, being not medically least important, is neglected by health and health related organizations and researchers. The present finding (4.6%) is an alarming ring for the problem and alerts relevant bodies to how wide its distribution throughout the population is. Similar to pulmonary and other extra-pulmonary TB, the causative agent of GTB was also found to be *M. tuberculosis* in this study. Most patient, positive for endometrial TB, were not suspected by physical examination using clinical criteria. In the diagnosis of endometrial TB, IS1081-PCR was more sensitive and specific than histology, considering culture as gold standard.

The limitation of this study is that rates of FGTB prevalence cannot be accurately computed because the denominator, population base for the biopsied cases, is heterogeneous with regard to clinical presentations. The numbers of isolates are too few to provide a true picture of possible types of *M. tuberculosis* lineages. Comparison with histopathology is also hampered by lack of well-defined clinical selection criteria related to TB complaint.

In accordance with these conclusions, the following recommendations were forwarded.

1. Based on the finding, we recommend screening for GTB with well-designed studies to determine prevalence and assess the magnitude of the population affected and define risk groups.
2. We suggest that IS1081-PCR and/or culture are a good candidate assays for screening of GTB in clinically indicated situations. We encourage complementing histology with IS1081-PCR. We recommend further validation of our finding that it appears to be more sensitive and specific, and has a shorter turn-around time with the result available earlier.
3. Providing diagnostic support and facilities that help physicians in the diagnosis of GTB is also highly recommended.

Reference

Abebe, M., Lakew, M., Kidane, D., Kirose, K. & Harboe, M. 2004. Female genital Tuberculosis in Ethiopia. *Int J Gynaecol Obstet.,* **84**, 241-246.

Aranaz, A., Liebana, E., Gomez-Mampaso, E., Galan, J. C., Cousin, D., Ortega, A., Blazquez, J., Baquero, F., Mateos, A., G., S. & Dominguez, L. 1999. Mycobacterium tuberculosis su bsp. caprae subsp. nov.: a taxonomic study of a new member of the Mycobacterium tuberculosis complex isolated from goats in Spain. *Int J Syst Bacteriol.,* **49**, 1263-1273.

Bahador, A., Etemadi, H., Kazemi, B., Ghorbanzadeh, R., Nakhjavan, F. A. & Nejad, Z. A. 2005. Performance Assessment of IS1081-PCR for Direct Detection of Tuberculous Pleural Effusion:Compared to rpoB-PCR. *Res. J. Agric. & Biol. Sci.,* **1**, 142-145.

Baxi, A., Neema, H., Kaushal, M., Sahu, P., and Baxi, D. 2011. Genital Tuberculosis in infertile women: Assessment of endometrial TB PCR result with laparascopic and hysteroscopic features. J Obtstet Gynecol India, **61**, 301-306.

Behr, M. A. 2002. different strains, different vaccines? *Lancet Infect.Dis.,* **2**, 86–92.

Behr, M. A., Wilson, M. A., Gill, W. P., Salamon, H., Schoolnik, G. K., Rane, S. & M., S. P. 1999. Comparative genomics of BCG vaccines by whole- genome DNA microarray. *Science* **284**, 1520–1523.

Bhanu, N. V., Singh, U. B., Chakraborty, M., Suresh, N., Arora, J., Rana, T., Takkar, D. & Seth, P. 2005. Improved diagnostic value of PCR in the diagnosis of female genital tuberculosis leading to infertility. *J Med Microbiol,* **54**, 927-31.

Boddinghaus, B., Rogall, T., Flohr, T., Blocker, H. & Bottger, E. C. 1990. Detection and identification of mycobacteria by amplification of rRNA. *J. Clin. Microbiol.,* **28**, 1751-1759.

Brennan, M. J. &Thole, J. 2012. Tuberculosis vaccines: a strategic blueprint for the next decade. Tuberculosis (Edinb), **92**, 6-13.

Brosch, R., Gordon, S. V., Marmiesse, M., Brodin, P., Buchrieser, C., Eiglmeier, K., Garnier, T., Gutierrez, C., Hewinson, G., Kremer, K., Parsons, L. M., Pym, A. S., Samper, S., Van Soolingen, D. & Cole, S. T. 2002. A new evolutionary scenario for the Mycobacterium tuberculosis complex. *Proc Natl Acad Sci U S A,* **99**, 3684-3689.

Calmette, A. & Gue´Rin, C. 1909. The primary mechanism of attenuation of bacillus Calmette–Guérin *C. R. Acad. Sci,* **149**, 716–718.

Chavhan, G. B., Hiva, P., Rathod, K., Zacharia, T. T., Chawla, A., Badhe, P. & Parmar, H. 2004. Female genital tuberculosis: Hysterosalpingographic appearances. *Br J Radiol,* **77**, 164-169

Cole, S. T., Brosch, R., Parkhill, J., T., G., Churcher, C., Harris, D., Gordon, S. V., Eiglmeier, K., Gas, S., Barry Iii, C. E., Tekaia, F., Badcock, K., Basham, D., Brown, D., Chillingworth, T., Connor, R., Davies, R., Devlin, K., Feltwell, T., Gentles, S., Hamlin, N., Holroyd, S., Hornsby, T., Jagels, K., Krogh, A., Mclean, J., Moule, S., Murphy, L., Oliver, K., Osborne, J., Quail, M. A., Rajandream, M. A., Rogers, J., Rutter, S., Seeger, K., Skelton, J., Squares, R., Squares, S., Sulston, J. E., Taylor, K., Whitehead, S. & Barrell, B. G. 1998. Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. *Nature,* **393**, 651-653.

Collins, D. M. & Stephens, D. M. 1991. Identification of insertion sequence, IS1081, in Mycobacterium bovis. *FEMSLett,* **83**, 11-16.

Cousins, D., Bastida, A., Cataldi, V., Quse, S., Redrobe, S. & Dow, P. 2003. Tuberculosis in seals caused by a novel member of the Mycobacterium tuberculosis: Mycobacterium pinnipedii sp.nov. . *Int. J. Syst. Evl. microbiol.,* **53**, 1305-1314.

Dannenberg, A. M., Jr. 1989. Immune mechanisms in the pathogenesis of pulmonary tuberculosis. *Rev. Infect. Dis.,* **11**, S369–S378.

Derese, Y., Hailu, E., Assefa, T., Bekele, Y., Mihret, A., Aseffa, A., Hussien, J., Ali, I. & Abebe, M. 2012. Comparison of PCR with standard culture of fine needle aspiration samples in the diagnosis of tuberculosis lymphadenitis. *J Infect Dev Ctries,* **6**, 53-57.

Devynck, W. E. & Kruger, T. F. 1990. Associated with female infertility in West Cape. *S Afr Med J,* **77**, 630-631.

Fleischmann, R. D., Dodson, R., Ermolaeva, M., Alland, D., Gwinn, M., Haft, D., Salzberg, S. L., Mikula, A., W. Bishai, Eisen, J. A., Hickey, E., Delcher, A., Carpenter, L., Kolonay, J. F., Utterback, T., Jacobs, J., W. R. , White, O., Peterson, J., Nelson, W. C., Weidman, J., Venter, J. C., Deboy, R., Umayam, L. A., Khouri, H., Mikula, A., Bishai, W., Gill, J. & Fraser, M. 2002. Whole-Genome Comparison of Mycobacterium tuberculosis Clinical and Laboratory Strains. *J. Bacteriol.,* **184**, 5479–5490.

Gordon, S., Brosch, R., Billaut, T., Garnier, K., Eiglmeier & Cole, S. T. 1999. Identification of variable regions in the genomes of tubercle bacilli using bacterial artificial chromosome arrays. *Mol Microbiol.,* **32**, 643-655.

Groenen, P. M., Bunschoten, A. E., Van Soolingen, D. & Van Embden, J. D. 1993. Nature of DNA polymorphism in the direct repeat cluster of Mycobacterium tuberculosis; application for strain differentiation by a novel typing method. *Mol Microbiol.,* **10**, 1057-1065.

Halpern, M., Castineiras, M., Souza, S., Hofer, E. & Martins, S. 1994. Cavitary pneumonia caused by Rhodococcus equi in patient with AIDS. *Int Conf AIDS,* **10**, 181 (abstract no. PB0739).

Henderson, R. A., Watkins, S. C. & Flynn, J. L. 1997. Activation of human dendritic cells following infection with Mycobacterium tuberculosis. *J Immunol.,* **159**, 635-643.

Hillemann, D., Rusch-Gerdes, S. & Richter, E. 2007. Evaluation of the GenoType MTBDRplus assay for rifampin and isoniazid susceptibility testing of Mycobacterium tuberculosis strains and clinical specimens. *J Clin Microbiol.,* **51**, 2635-2640.

Hillemann, D., Rusch-Gerdes, S. & Richter, E. 2009. Feasibility of the GenoType MTBDRsl assay for fluoroquinolone, amikacin-capreomycin, and ethambutol resistance testing of Mycobacterium tuberculosis strains and clinical specimens. *J Clin Microbiol.,* **47**, 1767-1772.

Huard, R. C., Fabre, P., De Haas, L., Lazzarini, C., Van Soolingen, D. & Cousins, D. 2006. Novel genetic polymorphisms that further delineate the phylogeny of the mycobacterium tuberculosis complex. *J. Bacteriol.,* **188**, 4271-87.

Huard, R. C., Lazzarini, L. C., Butler, W. R., Van Soolingen, D. & Ho, J. L. 2003. PCR-based method to differentiate the subspecies of the Mycobacterium tuberculosis complex on the basis of genomic deletions. *J Clin Microbiol.,* **41**, 1637-1650.

Jahromi, N. B., Parsanezhad, M. E. & Ghane-Shirazi, R. 2001. Female genital tuberculosis and infertility. *Int J Gynaecol Obstet.,* **75**, 269-272.

Jain, A. 2011. Extra Pulmonary Tuberculosis: A Diagnostic Dilemma. *Ind J Clin Biochem,* **26,** 269–273.

Jindal, U. N. 2006. An algorithmic approach to female genital tuberculosis causing infertility. *Int J Tuberc Lung Dis,* **10**, 1045-1050.

Kallenius, G., Koivula, T., Ghebremichael, S., Hoffner, S. E., Norberg, R., Svensson, E., Dias, F., Marklund, B. I. & Svenson, S. B. 1999. Evolution and clonal traits of Mycobacterium tuberculosis complex in Guinea-Bissau. *J Clin Microbiol.,* **37**, 3872-3878.

Kamerbeek, J., Schouls, L., Kolk, A., Van Agterveld, M., Van Soolingen, D., Kuijper, S., Bunschoten, A., Molhuizen, H., Shaw, R., Goyal, M. & Van Embden, J. 1997. Simultaneous detection and strain differentiation of Mycobacterium tuberculosis for diagnosis and epidemiology. *J Clin Microbiol.,* **35**, 907-914.

Katoch, M. 2004. Infections due to non-tuberculous mycobacteria. *Indian J Med Res.,* **120**, 290-304

Kohli, M. D., Nambam, B., Trivedi, S. S., L., S. B., Arora, S. & Jain, A. 2011. PCR-Based Evaluation of Tuberculous Endometritis in Infertile Women of North India. *J Reprod Infertil.,* **12**, 9-14.

Kubica, G. P., Dye, W. E. & Cohn, M. L. 1964. Sputum digestion and decontamination with N-acetyl-L-cysteine-sodium hydroxide for culture of mycobacteria. *Am Rev Respir Dis.,* **89**, 284-286.

Kulshrestha, V., Kriplani, A., Agarwal, N., Singh, U. B. & Rana, T. 2011. Genital tuberculosis among infertile women and fertility outcome after antitubercular therapy. *Int J Gynaecol Obstet.,***113**, 229–234.

Lee, E. & Holzman, R. S. 2002. Evolution and current use of the tuberculin test. *Clin Infect Dis.,* **34**, 365–370.

Liu, J., Tran, V., Leung, A. S., Alexander, D. C. & Zhu, J. L. B. 2009. BCG vaccines Their mechanisms of attenuation and impact on safety and protective efficacy. *Hum. Vaccin.,* **5**, 70–78.

Madhu, N. & Davinder, P. 2000. Genital Tuberculosis: Present Scenario. *JK Sciece,* **3**, 20-25.

Mahairas, G. G., Sabo, P. J., Hickey, M. J., Singh, D. C. & Stover, C. K. 1996. Molecular analysis of genetic differences between Mycobacterium bovis BCG and virulent M. bovis. *J. Bacteriol.,* **178**, 1274–1282.

Margolis, K., Wranz, P., Kruger, T. F., Joubert, J. J. & Odendaal, H., J. 1992. Genital tuberculosis at Tygerberg Hospital – prevalence, clinical presentation and diagnosis. *S Afr Med J.,* **81**, 12–15.

Mathema, B., Kurepina, N. E., Bifani, P. J. & Kreiswirth, B. N. 2006. Molecular Epidemiology of Tuberculosis: Current Insights. *Clin.Microbiol.Rev.,* 19**,** 658–685.

Mazurek, G. H., Lobue, P. A. & Daley, C. L. 2001. Comparison of a wholeblood interferon gamma assay with tuberculin skin testing for detecting latent Mycobacterium tuberculosis infection. *J Am Med Asso.,* **286**, 1740–1747.

Michael, T. G., Danny, R. W., Palmer., S., Keith, J. & Hewinson, G. R. 2007a. Rapid detection of Mycobacterium bovis DNA in cattle lymph nodes with visible lesions using PCR. *BMC Veterinary Research,* **3**, 1-12.

Michael, T. G., Yuri, C., Eileen, M., Richard, H. & Paul, R. 2007b. First report of Mycobacterium bovis DNA in human remains from the Iron Age. *Microbiology,* **153**, 1243–1249.

Mukantabana, S. 1985. Infertility in Rwanda. *Imbonezamuryang,* **2**, 21-27

Niemann, S., Richter, E. & Rusch-Gerdes, S. 2000. Differentiation among Members of the Mycobacterium tuberculosis Complex by Molecular and Biochemical Features: Evidence for Two Pyrazinamide-Susceptible Subtypes of M. bovis. *J. C. Microbiolo.,* **38**, 152–157.

Nogales-Ortiz F, E. A. 1979. Pathology of female genital tuberculosis: a 31 year study of 1436 cases. *Obstet Gynecol.,* **53**, 422–428.

Normung, D. I. F. 1986. Medical Microbiology: Diagnosis of Tuberculosis. Part 9: Minimum Requirements for the Identification of the Tubercle Bacilli. *DIN,* Beuth Verlag, Berlin, Germany**,** 58943-58949.

Othieno, E., Odida, M., Gemagaine, G., Okwi, A., Bimenya, G. S. & Wandabwa, J. 2008. Female genital tuberculosis in Uganda. *AJABS,* **30**, 30-34.

Pan, Y., Yang, X., Duan, J., Lu, N., Leung, A. S., Tran, V., Hu, Y., Wu, N., Liu, D., Wang, Z., Yu, X., Chen, C., Zhang, Y., Wan, K. & Zhu, J. L. B. 2011. Mycobacterium bovis BCG Vaccine Strains Whole-Genome Sequences of Four. *J. Bacteriol.,* **193**, 145-152.

Parikh, F. R., Nadkarni, S. G. & Kamat, S. A. 1997. Genital tuberculosis a major pelvic factor causing infertility in Indian women. *Fertile Steril.,* **67**, 497–500.

Pulimood, A. B., Shajan, P. Rook, G. W.A., Donoghue, H. D. 2008. In Situ PCR for Mycobacterium tuberculosisin Endoscopic Mucosal Biopsy Specimens of Intestinal Tuberculosis and Crohn Disease. Am J Clin Pathol., 129, 846-851.

Rastogi, N., Legrand, E. & Sola, C. 2001. The mycobacteria: an introduction to nomenclature and pathogenesis. *Rev. sci. tech. Off. int. Epiz.,* **20**, 21-54.

Reif, H. J. & Saedler, H. 1975. IS1 is involved in deletion formation in the gal region of E. coli K12. *Mol. Gen. Genet.,* **137**, 17–28.

Rieder, H. L., Van Deun, A., Kam, K. M., Kim, S. J., Chonde, T. M., Trébucq, A. & Urbanczik, R. 2007. Priorities for tuberculosis bacteriology services in low-income countries. *IUALTD, Paris, France.* 2 edn.

Roach, D. R., Bean, A. G., Demangel, C., France, M. P., Briscoe, H. & Britton, W. J. 2002. TNF regulates chemokine induction essential for cell recruitment, granuloma formation, and clearance of mycobacterial infection. *J Immunol.,* **168**, 4620-4627.

Saracoglu, O. F., Mungan, T. & Tanzer, F. 1992. Pelvic tuberculosis. *Int J Gynaecol Obstet.,* **37**, 115–120.

Sathe, A. V., Vaidya, P. R., Deshmukh, M. A. & Motashaw, N. D. 1979. Genital tuberculosis in an endocrine clinic. *J Obstet Gynaecol India,* **29**, 199-202.

Sharma, S. K., Mohan, A., Sharma, A. & Mitra, D. K. 2005. Miliary tuberculosis: new insights into an old disease. *Lancet Infect Dis,* **5**, 415–430.

Singleton, P. & Sainsbury, D. 2006. Dictionary of Micro-Biology And Molecular Biology. Third Edition.

Sreevatsan, S., Pan, X., Stockbauer, K. E., Connell, N. D., Kreiswirth, B. N., Whittam, T. S. & Musser, J. M. 1997. Restricted structural gene polymorphism in the Mycobacterium tuberculosis complex indicates evolutionarily recent global dissemination. *Proc Natl Acad Sci USA.,* **94**, 9869-9874.

Sutherland, A. M. 1983. The changing pattern of tuberculosis of the female genital tract. A thirty-year survey. *Arch Gynecol.,* **234**, 95–101.

Swaminathan, S., Padmapriyadarsini, C. & Narendran, G. 2010. HIV-Associated Tuberculosis: Clinical Update. *Clin Infect Dis.,* **50**, 1377–1386.

Thangappah, R. B. P., Paramasivan, C. N. & Narayanan, S. 2011. Evaluating PCR, culture & histopathology in the diagnosis of female genital tuberculosis. *Indian J Med Res.,* **134**, 40-46.

Thierry, D., Brisson-Noël, A., Vincent-Lévy-Frébault, V., Nguyen, S., Guesdon, J. & Gicquel, B. 1990. Characterization of a Mycobacterium tuberculosis Insertion Sequence, IS6110, and Its Application in Diagnosis. *J. C. Microbiolo.,* **28**, 2668-2673

Thomas, R. F., Sterling, T. R., Munsiff, S. S., Watt, C. J. & Dye, C. 2003. Tuberculosis. *The Lancet,* **362**, 887-899.

Tripathy, S. N. & Tripathy, S. N. 1998. Gynaecological Tuberculosis-An Update. *Leading Article,* **193**, 182-185.

Truffot-Pernot, C., Véziris, N. & Sougakoff, W. 2006. Modern diagnosis of tuberculosis. . *Presse Med.,* **35**, 1739-1746.

Turner, O. C., Basaraba, R. J. & Orme, I. M. 2003. Immunopathogenesis of Pulmonary Granulomas in the Guinea Pig after Infection with Mycobacterium tuberculosis. *Infect.Immun.,* **71**, 864–871.

Udwadia, Z. F., Amale, R. A., Ajbani, K. K. & Camilla, R. 2012. Totally drug-resistant tuberculosis in India. *Clin Infect Dis.,* **54**, 579e81.

Van Embden, J. D., Van Gorkom, T., Kremer, K., Jansen, R., Van Der Zeijst, B. A. & Schouls, L. M. 2000. Genetic variation and evolutionary origin of the direct repeat locus of Mycobacterium tuberculosis complex bacteria. *J Bacteriol.,* **182**, 2393-2401.

Van Soolingen, D. & De Haas, P. H. T. 1997. A novel pathogenic taxon of the Mycobacterium tuberculosis complex, Canetti: characterization of an exceptional isolate from Africa. *Int J Syst Bacteriol.,* **47**, 1236–1245.

Van Soolingen, D., Hermans, P. W., De Haas, P. E. & Van Embden, J. D. 1992. Insertion element IS1081-associated restriction fragment length polymorphisms in Mycobacterium tuberculosis complex species: a reliable tool for recognizing Mycobacterium bovis BCG. *J Clin Microbiol.,* **30**, 1772-1777.

Varma, T. R. 1991. Genital tuberculosis and subsequent fertility. *Int J Gynecol Obstet.,* **35**, 1–11.

Vynnycky, E. & Fine, P. E. 1997. The natural history of tuberculosis: the implications of age-dependent risks of disease and the role of reinfection. *Epidemiol Infect.,* **119**, 183-201.

Vynnycky, E., Nagelkerke, N., W., B. M., Van Soolingen, D., Van Embden, J. D. A. & Fi Ne, P. E. M. 2001. The effect of age and study duration on the relationship between ` clustering ' of DNA fingerprint patterns and the proportion of tuberculosis disease attributable to recent transmission. *Epidemiol. Infect.,* **126**, 43-62.

Wayne, L. G., Good, R. C., Krichevsky, R. C., Blacklock, Z., David, H. L., Dawson, D., Gross, W., Hawkins, J., Levy-Frebault, V. V., Mcmanus, C., Portaels, F., Ru¨Sch-Gerdes, S., Schro¨Der, K. H., Silcox, V. A., Tsukamura, M., Breen, L. V. & Yakrus, M. A. 1991. Fourth report of the cooperative, open-ended study of slowly growing mycobacteria by the International Working Group on Mycobacterial Taxonomy. *Int. J. Syst. Bacteriol.,* **41**, 463–472.

Weinberg, A. C. & Boyd, S. D. 1988. Short-course chemotherapy and role of surgery in adult and pediatric genito-urinary tuberculosis. *Urology,* **31**, 95-98.

WHO. 2009. Guidelines for surveillance of drug resistance in tuberculosis. *WHO/HTM/TB***,** 422.

WHO. 2010. Multidrug and extensively drug-resistant TB (M/XDR-TB) : 2010 global report on surveillance and response. *WHO/HTM/TB/2010.3 ed. World Health Organization, Geneva, Switzerland*.

WHO. 2012. Global Tuberculosis Control. *WHO Report*.

WHO & TB, S. 2010. The global plan to stop TB 2011-2015: transforming the fight towards elimination of tuberculosis. *World Health Organization, Geneva.*

WHO/HTM/TB. 2009. Treatment of tuberculosis: guidelines. *4th ed.,* 420.

Annex

Annex 1: Participant Information Sheet

Date ----------

Title: Prevalence of Endometrial Tuberculosis and Characterization of Isolates among patients undergoing endometrial biopsy at Tikur Anbesa Specialized Hospital, Addis Ababa, Ethiopia

Background: Tuberculosis is an infectious disease that causes considerable morbidity and mortality. Globally, 8.4 million people are estimated to develop tuberculosis each year, and arly 2 million deaths result from the disease. Overall, one-third of the world's population is currently infected with the tuberculosis bacillus, over 90 per cent of them in developing countries. Ethiopia ranks seventh among the world's 22 high-burden tuberculosis countries. The prevalence of genital tuberculosis (GTB) increases in countries with high pulmonary (PTB) burden.

Objective of the study: To determine the prevalence of endometrial tuberculosis and characterization of isolates among patients undergoing endometrial biopsy, and to compare the result of TB suspected patients with histo-pathological result. The sensitivity and specificity PCR DNA extraction will be compared against culture method.

Organizations: The study will be conducted by Addis Ababa University, school of Graduate studies and Armauer Hanson Research Institute (AHRI/ALERT). Laboratory procedure is to be carried out at Akililu Lemma Institute of Patho-Biology (culture) and AHRI (DNA extraction).

Procedure: Expert (in the area) physicians will collect endometrial biopsy for routine hito-pathological diagnosis. If there is leftover sample, it will be collected by assistants (Nurses), for the research purpose. The procedure will not be repeated if no extra sample is left, and you will be excluded from the study. Laboratory method to be used includes; culture, Genotyping, Deletion Typing, Spoligotyping, and PCR DNA extraction.

Participation: The procedure is to be carried out after getting your willingness to participate. All volunteer patients with endometrial problem, fulfilling inclusion criteria, will be included.

Risks associated with sample collection: You will experience pain during specimen collection, although that is not incurred because of participation in this study. However, you will be treated accordingly. To reduce the pain, you will be administered local anesthetics.

Benefit: As different study shows, most of the time, GTB is symptomless and end up with irreversible damage (e.g. infertility). You will be benefited from the study; because it will be part of your diagnosis and might be a key to your current and/or your future problem if it will come up with positive result.

Compensation: You will receive your result (only positive patients) through your physician. You will get treatment for free if you become positive for endometrial TB. Fees for transport and elapsed time will also be compensated.

Confidentiality: From medical ethics point of view and research ethics, every part of your personal information will be kept confidentially. Information to be collected and

variables expressing your identity will be coded secretly. The only responsible person to link your variables (important for your follow up and treatment) with the code number is the principal investigator. However, other researchers can see your clinical information, which is without your identity. Your result and information will be used only for the mentioned purpose.

Sharing the Result: Eventually, the result, devoid of your identity, will be reported through publication or by other means. Have no suspicion on the confidentiality of your information, even at this time. We request your permission to use the result for reporting.

Right to refuse or withdraw: Thus, it is your right to agree or to refuse to participate in the study. Withdrawal from the study is also possible, at any time. Withdraw or refusing to participate will not have any impact on your normal diagnosis or medical follow up. You can address your problem or question through one of the addresses given bellow.

Contact Addresses:

1. **Sileshi Abdissa,** Address: Addis Ababa University, College of Health Science, department of Medical Microbiology, Immunology and Parasitology, Mobile: +251913216087, E-mail – silejes@gmail.com
2. **Tamirat Abebe,** Address: Addis Ababa University, College of Health Science, department of Medical Microbiology, Immunology and Parasitology, Mobile: +251911447227, E-mail tabebezeleke@yahoo.com

3. **Dr. Gobena Ameni** (PhD, Associate Professor), Address: Aklilu Lemma Institute of patho-biology (ALIPB), Addis Ababa University, Ethiopia, mobile: - +251-911413073, E-mail: - gobenachimdi2009@yahoo.co.uk

4. **Dr. Markos Abebe** (post doc scientist), Address: Armauer Hanson Research Institute (AHRI), Addis Ababa, Ethiopia
   Mobile: - +251-911744583, E-mail: - markosabebe@yahoo.com
5. **Dr. Sisay Teklu** (gynecology and obstetric), address: Addis Ababa University, College of Health Science, department of gynecology and obstetric. Mobile +251911227806, E-mail
6. **Addis Ababa University (College of Health Science),** address: Office of Associate Dean, Postgraduate Programs and Research. Tel. + 251-011-551-28-765, P.O. Box 9086, Addis Ababa, Ethiopia

7. **AHRI/ALERT Ethics Review Committee (AAERC),** address: Tel. +251-113-211567, **P.O.Box 1005,** Addis Ababa, Ethiopia

Thank you for your patience and kindness

ANNEX 2: Informed Consent Form

Name ----------, Age ----------, Sex -------

Identification No ----------, lab No ----------

I read and/or well informed about the nature of the study, entitled **"Prevalence of Endometrial Tuberculosis and Characterization of Isolates among patients undergoing endometrial biopsy at Tikur Anbesa Specialized Hospital, Addis Ababa, Ethiopia"**. Though not due to my involvement in the study, I will experience pain during the procedure and only leftover sample is to be used. Then, she/he asked my willingness to allow the leftover sample to be collected for the research purpose. Finally, she/he told me that this will be certain if I agree on the following points and signed bellow.

a) I understood the objective of the study
b) I understood that the sample won't be used for farther study, and after completion of the whole procedure, the leftover sample will be discarded safely
c) I am aware of any information describing my identity, collected using questionnaire and , won't be disclosed
d) I understood report of my result won't include my name
e) I understood that I won't get money for being part of the study except compensation for transportation and elapsed time if only the result become positive.
f) I clearly informed as I have the right to refuse to participate and withdraw (if I change my idea) from the study at any time

g) I understood that my refusal to take part in this study won't have impact to the normal diagnosis and to my future medical follow up.

I have had enough time to think over it freely and I understood it well. I found it would have positive impact in the investigation of my case. My agreement to this consent is without any external enforcement, and will be confirmed by my signature, below.

The information sheet was given/ explained to me by: ----------, signature ---------, phone ----------

Name of participant: ----------, signature ----------, phone ------Name of physician: ----------, signature --------, phone, ----------

ANNEX 3: Declaration Sheet

Titleof the study: Prevalence of Endometrial Tuberculosis and Characterization of Isolates among patients undergoing endometrial biopsy at Tikur Anbesa Specialized Hospital, Addis Ababa, Ethiopia

I prioritize the patient's safety and I am responsible for the role(s) I am going to play.

Investigators	Role(s)	signature	Remark
Sileshi Abdissa (Mathema et al.)	Write up Material supply Sample care and transportation Processing the whole lab, principally		
Tamirat Abebe	Supervising the whole process		
Dr Markos Abebe	Supervising the lab part Write up Material supply		
Dr Sisay Teklu	Sample collection Treatment to the participant Clinical information of the participant Write up		

Annex 4. Questionnaire

Part 1		
Variables	**Coding categories**	Remark
1.1. Name		
1.2. Identification number		
1.3. Age		
1.4. Are you from Addis Ababa?	A) Yes B) No, specify ……	
1.5. Marital status	A) marred B) not marred C) Divorced D) widowed	
1.5. Have you borne child?	A) Yes B) No C) Aborted	
1.6. Are you able to bear child now (for patients before menopause, < 47 years).	A) Yes B) No	
.7. If not for question #5, specify the year you stopped.	Before -----	
.8. Have you ever encountered menstrual disturbance?	A) Yes: 1. Menorrhagia 2. Amenorrhea 3. Oligomenorrhea B) No	
.9. Did you feel chronic pelvic pain?	A) Yes, B) No	
.10. Have you been treated with anti-TB drug in the past?	A Yes(before-----year/s) B) Currently C) No	
Part 2:- To be Filled by the Interviewer		
2.1. Has the patient been suspected for endometrial TB, by the physician?	A) Yes B) No	
2.2. Result of HIV screening	A) Positive B) Negative C) Not done	
2.3. Investigation is for:	A) Infertility (2^0, 1^0) B) Others	

ለተሳታፊዎች የጥናቱ መረጃ ቅፅ

ቀን ----------

ርዕስ፡ "የቲቢ በሽታ ስርጭትና የተዋህስያኑ አይነት በሰቶች ማህፀን ላይ በጥቁር አንበሳ ሆስፒታል"

መግቢያ፡. ቲቢ ፣ ማይኮ ባክተሪዬም በምባል ተዋህስያን የምመጣ ስሆን ፤ ብዙ ህመምና ሞትን እያደረሰ ያለ በሽታ ነዉ፡፡ በዓለም ላይ በዓመት 8.4 ምልዮን ህዝብ እነደምያዝና ፤ 2 ምልዮን የምሆኑ ደግሞ እነደምሞቱ ይገመታል፡፡ በአጠቃላይ ፣ በአሁኑ ጊዜ 1/3 የአለም ህዝብ በቲቢ ባክተሪያ የተጠቃ ነዉ ፣ ዘጠና ከመቶ በላይ በታዳጊ ሀገሮች ይገኛሉ፡፡ በአለም ላይ ቲቢ በብዛት ከምገኝባቸዉ 22 ሀገሮች ፤ ኢትዮጵያ በ 7ተኛ ደረጃ ላይ ትገኛለች፡፡ የሰቶች በመራብያ አካል ቲቢ የመያዝ ዕድልም የሳንባ ቲቢ በምበዛባቸዉ ሀገራት ላይ ይጨምራል፡፡

የጥናቱ ዓላማ፡ በሰቶች ማህፀን ዉስጥ ያለዉን የቲቢ ስርጭት መጠንን ለማወቅና ተዋህስያኑን ለመለየት፡፡ በፓቶሎጂና አሁን በምጠቀመዉ መሳሪያ መካከል ያለዉን የመለየት ብቃት ለማወዳደርና የፕስ አር/PCR ባክተሪያዉን የመለየት ችሎታ ለማወቅ፡፡

ጥናቱን የምያካህዱ ድርጅቶች፡ ኣድስ አባባ ዩንቨርሲቲ ፣ የድህረ ምረቃ ትምህረት ቤትና አርማወር ሀንሰን የምርመር ተቋም ነዉ፡፡ የላቦራቶሪዉ ስራ የምካሄድበት ቦታዎች ፣ በአከሊሉ ለማ መካነ-ጥናት የምርምር ተቋምና በአርማወር ሀንሰን የምርምር ተቋም ነዉ፡፡

የናሙና አወሳሰድ ህደት፡ በሙያዉ የተካኑ ሀኪሞ ለታዘዘሎት ለፓቶሎጂ ምርመራ ፤ ናሙናዉን ይወስዳሉ፡፡ ረዳቱ/ቷ ነርስ ለታዘዘሎት ምርመራ ናሙናዉን ከሰበሰቡ በኋላ ፤ የተረፈ ናሙና ካለና እርስዎ ፈቅደዉ ከሆኔ ለጥናቱ አላማ ይሰበስባሉ፡፡ ነገር ግን የተረፈ ናሙና ከለለ ፤ ለጥናቱ አላማ ተብሎ ናሙና እነደገና አይወሰድም ::

ተሳትፎ፡ በጥናቱ ዉስጥ ያለመሳተፍ መብትዎ የተጠበቀ ነዉ :: ናሙናዉን የምንወስደዉ የእርስዎን ሙሉ ፍቃድ ካገኘን ብቻ ነዉ፡፡ መስፈርቱን የምያሚሉና ፍቃደኛ የሆኑ ሴቶች ሁሉ በጥናቱ ዉስጥ ይገባሉ፡፡

በናሙና አወሳሰድ ጊዜ ልየጋጥሞት የምችል ችግር፡ በዝህ ጥናት ዉስጥ ስለተሳተፉ ባይሆንም ናሙና በምወሰዲሎት ጊዜ ህመም ይሰማዎታል፡፡ ይሁን እንጅ በተቻለ መጠን ጥንቃቄ ይደረጋል ፤ ህመሙን ለመቀነስ ማደንዘዣም ይሰጦታል፡፡

ከጥናቱ ልያገኙ የምችሉ ጥቅም፡ ጥናቶች እንደምያሳዩት ፣ ብዙዉን ጊዜ ፣ የመራብያ አካል ቲቢ ምልክት ሳያሳይ ልድን ለማይቻል ለአካል ጉዳት ይዳሪጋል (ለምሳሌ መካንነት)፡፡ ትናቱ የምርመራዎ አንድ አካል ስለሆኔ ፤ የቲቢ ባከተሪያ ከተገኘቦት ፣ አሁን ላሉበት ችግር መፍተሄ ልሆንዎ ይችላል፡፡

ማካካሻ፡ የቲቢ ባከተሪያ ከተገኘቦት ብቻ ወደ ህክምዎ ቀርበዉ መዲህንት በነጻ ያገኛሉ ፡፡ መዲሁንቱን ለመዉስድ ስመጡ ፤ ለትራንስፖርት ላወጡት ገንዘብና ላባከኑት ጊዜ ተመጣጣኝ ገንዘብ ይሰጦታል፡፡

ምስጥር ስለ መጠበቅ፡ በህክምና ሙያም ሆነ በምርምር ስራት መሰረት፣ ማንኛዉም ዓይነት የግል መረጃዎ በምስጥር ይጠቃል፡፡ በመጠየቅ የተሰበሰበዉ መረጃዎ ማንነትዎን በማይገልጽ በምስጥር ኮድ ወደ ኮምፕተር እንድገባ ይደረጋል፡፡ የላቦራቶሪ ዉጠትዎም ምስጥሩ የተጠበቀ ከመሆኑም በላይ የእርስዎን ስም አይዝም፡፡ ይሁን እንጂ፣ ለሎች ተመራማሪዎች የርስዎ ማንነት የለለበትን ዉጠትዎን ልያዩ ይችላሉ፡፡ መረጃዎና ዉጠትዎ ለተጠቀሰዉ አላማ ብቻ ይዉላል፡፡

የዉጠት አገላለፅ ህደትና መንገድ፡ ጥናቱ ከተጠናቀቀ በኃላ ፣ ዉጠቱ በተለያየ መንገድ ይገለፃል፡፡ የእርስዎን ማንነት እነደመይዝና ምስጥርዎ እነደ ተጠበቀ ይሆናል፡፡ ለዝህም የእርስዎን በጎ ፍቃድ እንጠይቃለን፡፡

በጥናቱ ያለመሳተፍ መብት፡ በጥናቱ ዉስጥ የመሳተፍም ሆኔ ያለ መሳተፍ ሙሉ መብት አለዎት፡፡ ናሙናዉን ከፈቀዱ በኋላም ፣ በማንኛዉም ጊዜ ፣ ከጥናቱ ህደት ዉስጥ አቃርጠዉ መዉጣት ይችላሉ፡፡ በጥናቱ ዉስጥ አለመሳተፍም ሆኔ ማቋረጥ ፤ በምሰጥዎት ህክምና ላይ ምንም አይነት ችግር አያመጣም፡፡

ተመራማሪዎችን ልያገኙበት የምችሉብቸዉ አድራሻዎች፡ ስለ ጥናቱ ጥያቄ ካሎትም ሆኔ በምፈልጉበት ጊዜ ጥናቱን ማቋረጥ ከፈለጉ ፤ ከምከተሉት ዉስጥ የአንዱን አድራሻ ይጠቀሙ፡፡

1. **ስለሺ አብድሳ** ፣ አድራሻ :አድስ አባባ ዩኒቨረሲቲ፣ ሞባይል +251913216087 ፣ ኢ-መይል – silejes@gmail.com
2. **ታምራት አበበ** ፡ አድራሻ ፡ አድስ አበባ ዩንቨረሲቲ ሞባይል +251911447227 ኢ-መይል tabebezeleke@yahoo.com
3. **ዶር. ማረቆስ አበበ** (ፖስት ዶክተሪያል ሳይንትስት) **አድራሻ ፡** አረማወር ሀንሰን የምርምር ተቃም፤ አድስ አበባ ፣ ሞባይል +251-911744583 ኢ-ሜይል - markosabebe@yahoo.com
4. **ዶር. ጎበና አሜኒ** (ተባባሪ ፕሮፌሰር) **አድራሻ፡** አድስ አበባ ዩንቨርሲቲ፤ አክልሉ ሌማ መካነ ጥናት የምርምር ተቃም ፣ ሞባይል +251-911413073 ኢ-ሜይል - gobenachimdi2009@yahoo.co.uk

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የስምምነት መጠየቅ ቅፅ

ስም---------- ፣ ዕድሜ ---------፣ ፆታ ----------

የሆስፕታል ቁጥር ----------፣ የላቦራተሪ ቁጥር ----------

ካነበብኩት ወይም ከሰማሁት "የቲቢ በሽታ ስርጭትና የተዋህስያኑ አይነት በሰቶች ማህፀን ላይ በጥቁር አንበሳ ሆስፒታል" ስለ ተባለዉ ጥናት በቅ ግንዛቤ አግንቸአለሁ፡፡ ናሙናዉ ስወስድልኝም ፣ በጥናቱ ዉስጥ ስለተሳተፍኩ ባሆንም ፣ ህመም እነደምኖረዉ ተረድቻለሁ፡፡ የምወሰደዉ ናሙናም ከታዘዘልኝ ምረመራ የተረፈ መሆኑን ፤ ከለሌ ደግሞ ለጥናቱ ተብሎ እንደማይወሰድ ተገንዝበአለሁ፡፡ በመጨራሻም ናሙናዉን መፍቀደ የምረጋገጠዉ ፤ በምከተሉት ነጥቦች ከተሰማማሁ ብቻ መሆኑ ተነግሮኛል፡፡

ሀ. የጥናቱ ዓላማ ገብቶኛል

ለ. ናሙናዬም ሆነ መረጃዬ ለተገለጸዉ ዓላማ ወጪ እነደማይዉልና የተረፈ ናሙና ካለም በጥንቃቀ እነደምወገድ ተረድቻለሁ፡፡

ሐ. በመጠየቅ የስጠሁት መረጃዬም ሀነ የናሙናዉ ዉጠት ማንነተን እነደማይገልፅ ተረድቻለሁ፡፡

መ. በጥናቱ ተሳታፊ ስለሆንኩ ሳይሆን ፤ በባከትሪዉ ተጠቅ ከሆንኩ ብቻ የትራንሰፖርትና ላባከንኩት ጊዜ የምሆን ተመጣጣኝ ገንዘብ እንደምሰጠኝ አዉቀአለሁ፡፡

ሠ. በጥናቱ ዉስጥ የለመሳተፍም ሆነ ከገባሁ በኋላ የማቋረጥ መብት እንዳለኝ ተረድቻለሁ፡፡

ረ. በጥናቱ ዉስጥ ያለመሳተፍም ሆነ ማቋረጥ በምስጠኝ ህክምና ለይ ምንም ችግር እነደማያመጣ አዉቀአለሁ::

ስለ ጥናቱ ካነበብኩት ወይም ከሰማሁት ፣ በቂ ጊዜ ወስጄ ካሰብኩ በኋላ ፣ በቂ ማንዛቤ አገንቻለሁ:: ስለሆነም ፣ በጥናቱ ዉስጥ መሳተፈ ጠቃሚ ሆኖ ስላገኘሁት ፤ በራሰ ወስኘ ናሙናዉን እነድጠቀም መፍቀደን በፊርማዬ አረጋግጣለሁ::

የተሳታፊ ስም ---------- ፊርማ ----- ስልክ --------

የመርጃ ተቀባይ ስም ----------ፊርማ ------ ስልክ ---------

ናሙናዉን የወሰደዉ ህክም ---------- ፊርማ ---- ስልክ ----------

የመጠቅ ቅፅ

ክፍል አንድ		
መጠይቆች	**መለያ/መልሶች**	**ማሳሰብያ**
1.4. ስም ከነአባት		
1.5. መለያ ቁጥር		
1.3. እድሜ		
1.4. ከየት ነዉ የመጡት?	ሀ) አድስ አበባ ለ) ከአድስ አበባ ወጪ (ቦታዉን ይግለፁ) ……	
1.5. የጋብቻ ሁኔታ	ሀ) አግብቻለሁ ለ) አላገባሁም ሐ) ተለያይተናል መ) መበለት	
1.6. ልጅ ወልደዋል?	ሀ) ወልጀአለሁ ለ) አልወለደኩም ሐ) ዉርጃ	
1.7. አሁን መዉለድ ይችላሉ? (ዕድሜአቸዉ ከ 47 ዓመት በታች ለሆኑ እናቶች)	ሀ) አዎን ለ) አልችልም	
1.8. ለተራ ቁጥር 5 መልስዎ አልችልም ከሆነ ፤ መዉለድ ካቆሙ ስንት ዓመት ይሆኖታል?	------ ዓመት	
1.9.የወር አበባዎት በግዜ አለመምጣትንና የመጠን መለያየትን ያሳያል?	ሀ) አዎን 1. ብዙ ደም መፍሰስ 2. የመቅረት 3. ከተለመደዉ ግዜ በላይ መቆየት ለ) በትክለኛዉ ግዜ ይመጣል	
1.10. ለረጅም ግዜ የቆዬ የዳለ አከባብ ህመም ይሰማዎታል?	ሀ) አዎን ለ) አይሰማኝም	
1.11. ከዝህን በፍት የሳንባ ነቀረሳ መድሃንት ወስደዉ ያዉቃሉ?	ሀ) አዎን (ከ……..ዓመት በፍት) ለ) አልወሰድኩም	
ክፍል 2:- ቃለ መጠየቁን በምያካህደዉ የምሞላ		
2.1. ታማምዋ ለመራብያ አካል ነቀርሳ፣ በህክሙ፣ ተጠርጥረዋል?	ሀ) አዎን ለ) አልተጠረጠሩም	
2.2. የኤች አይቪ ምርምራ ዉጠታቸዉ፡	ሀ) ፖዝትቭ ለ) ነጌትቭ ሐ) አልተሰራም	
2.3ምረመራዉ ያስፍለገበት ምክንያት	ሀ) ለመካንነት (2^0 , 1^0) ለ) ለሌሎች	

DECLARATION

I, the undersigned, declare that this MSc research thesis is my original work. It has not been for a degree in any other university. False statements cause the invalidation of this research thesis and may lead to other administrative or legal actions.

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