MAKERERE UNIVERSITY

PREVALENCE AND FACTORS ASSOCIATED WITH SPUTUM SMEAR NEGATIVE TUBERCULOSIS AMONG ADULT TB PATIENTS IN KAWEMPE DIVISION-KAMPALA: A NESTED CASE CONTROL STUDY.

BY

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DECLARATION

I hereby declare that the work submitted in this Dissertation report is my own compilation and has not been submitted for another degree in this or any other university or institution of higher learning. All work is original unless otherwise acknowledge.

Sign……. ….……

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This dissertation has been submitted for examination with approval of the following supervisors:

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2. Dr. Mupere Ezekiel. (MBChB, MSc, M.MED, PhD)

Sign…… Date…….

DEDICATION

I dedicate this book to all those people who are infected and affected with TB and those who are still suffering from TB; they should know that “the best days are when you do not need anything extreme or special to happen to make it great. You just appreciate it and feel gratitude, seeking nothing else, nothing more, but God’s healing”.

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Lastly to my classmates thanks for your support spiritually and emotionally and may we continue to struggle together as we fight for the common goal.

May God bless you all

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LIST OF ACRONYMS

CWRU-MU	Case Western Reserve University-Makerere University Collaborations
HIV	Human Immunodeficiency Virus
AIDS	Acquired Immunodeficiency Syndrome
TB	Tuberculosis
MTB	Mycobacterium tuberculosis
DOTs	Directly Observed Therapy Short Course
WHO	World Health Organization

DEFINITION OF TERMS

AIDS: This referes to a progressive immune deficiency caused by infection of CD4+T cells with the human immunodeficiency virus (HIV).

Co-infection: The state of having other diseases apart from the HIV/AIDS infection

The diagnosis of tuberculosis (TB): Refers to the recognition of an active TB case: the identification of a patient who is symptomatic due to lesions caused by mycobacterium tuberculosis.

A TB case: Is a patient confirmed (by microbiologic studies) to harbor the organism mycobacterium tuberculosis. Infrequently, a case of TB may also be one where microbiologic work-up is negative but other data support or suggest the presence of the organism.

Smear negative tuberculosis: is currently defined as symptomatic illness in a patient with at least two sputum smear examinations negative for AFB on different occasions in whom pulmonary tuberculosis is later confirmed by culture, biopsy, or other investigations (WHO 2007).

ABSTRACT

Background

Smear microscopy has remained the most used test for diagnosis of tuberculosis in developing countries. However, there has been disproportionate increase in smear-negative tuberculosis. The objective of this study was to identify the prevalence and factors associated with smear-negative TB.

Methods: A cross sectional and case control study designs were in Mulago hospital, CWRU-MU treatment center. The clinical, demographic and other variables were extracted from adult TB patients' records, who were enrolled for treatment between 1st January 2002 and 31st December 2012 using a structured data extraction forms. Proportions and percentages were used to summarize categorical variables; and mean, median and standard deviation were used to summarize continuous variables. Bivariate and multivariate analyses (logistic regression) were used to identify clinical and socio-demographic features associated with smear-negative sputum.

Result: The overall prevalence of sputum smear-negative was 9.1%, 15.5% among HIV positive (OR= 1.87, 95% CI =1.026-3.398) and 10.9% among those who had history of previous use of anti-biotic (OR =1.76, 95% CI=1.039-3.422). Prevalence among those who were 36 years and above was 10.3%. The duration between collection of sample and laboratory analysis, (OR=1.23, 95% CI= 1.001-1.519), had significant relationship with sputum smear-negative tuberculosis. Being HIV positive (OR1.87, 95% CI 1.026-3.398), having had history of previous use of antibiotic (OR 1.76, 95% CI 1.039-3.442) and the X-ray (OR =0.54, 95% CI=0.278-0.785) were significantly associated with sputum smear-negative TB.

Conclusion: The prevalence of sputum smear-negative in this study was lower than the national level (26%). The sputum smear negative result was associated with HIV/AIDS and having history of previous use of antibiotic prior to sputum smear test. HIV positive TB patients were almost two

times more likely to have sputum smear-negative compared to HIV negative TB patients. Having had history of antibiotic use was almost twice more likely to give sputum smear-negative test result compared to those who never used antibiotic before the sputum smear.

CHAPTER ONE

1.0 Background

Tuberculosis (TB) is a major public health issue worldwide, particularly in low- and middle-income countries. It is estimated that one third of the world population is infected with Mycobacterium tuberculosis (WHO, 2011). Uganda is ranked 16th among the 22 high-burden countries that collectively account for 80% of TB cases globally, with an incidence of 38 cases per 100,000 inhabitants per year (WHO, 2011).

For decades, smear microscopy has remained the most used method for diagnosis of tuberculosis and it is the most feasible microbiological method for the diagnosis of TB in developing countries due to its rapidity, low cost, and high positive predictive value for Mycobacterium tuberculosis (Conde MB, et at, 2009). Many strategies like directly observed therapy of the short course (DOTs) use microscopic examination of sputum smears to identify infectious tuberculosis cases (acid-fast bacilli) (WHO, 2006a). However, with huge rises in incidence of tuberculosis in Uganda, partly due to HIV/AIDS (Fitz Gerald;Grzybowski; Allen EA, 1991), and low sensitivity of smear microscopy, there has been disproportionate increase in smear-negative tuberculosis. For example in Uganda, a total of 49,009 TB cases were notified and 25,448 (52%) of them were smear positive TB cases, while 12,824 were sputum smear negative (NTLP data, 2011) and the data on factors associated with increased risk of smear negative TB in Uganda are scarce. A study in Zambia on over 100 patients with culture positive TB, found that 24% of those who were HIV seronegative had a negative sputum smear, compared with 43% of those who were HIV seropositive (Elliott et al, 1993). With good routine reporting systems, the national tuberculosis programmes of countries such as Malawi and the United Republic of Tanzania have reported a larger increase in new cases of smear negative than of smear-positive TB in the last 10

years (Graf P, 1994). According to Colebunders and Bastian, smear negative TB account for 30-50% of TB (Colebunders R Bastian I, 2000) and causing significant morbidity and mortality particularly in high HIV-prevalence setting.

Much as microscopy is mostly used for the detection of acid-fast bacilli and is rapid, low cost, specific and detects the most infectious cases of tuberculosis, it needs maintenance of equipment, consistent supply of reagents, and proper training in interpretation of the slides. In addition, access to health services and DOTS in Uganda is restricted and services reach only a fraction of the population. If the availability of these services were increased, it is expected that a much higher frequency of TB would be seen and this would involve more testing to be conducted, putting more pressure on the already scarce resources. There would be more cases of negative smear TB which could be as a result of poor quality smear microscopy from inadequate sputum collection, storage, and staining, reading errors, or poor laboratory services.

Although chest radiography remains an important component of the diagnostic algorithm for smear-negative pulmonary tuberculosis, it can only be found in district, regional and national hospitals. Worse still, most of them, especially at district hospitals, are not working because either they are faulty or there are no personnel to operate them. Also the use of chest radiography for diagnosis of pulmonary tuberculosis can be compromised by poor film quality, low specificity, and difficulties with interpretation.

In Uganda health system structure, the diagnosis of TB starts from Health center II (HC II) which are poorly equipped and there are no arrangements for laboratory and other equipments. Therefore, the aim of this study was to identify the clinical, demographic, and radiographic

predictors for smear-negative TB to aid clinicians in predicting the likelihood of TB in persons with negative sputum smear results.

1.1 Problem Statement

Screening and early identification of TB, whether smear positive or smear negative, is important to enable both appropriate isolation procedures and provide a basis for early institution (Mfinanga G et al, 2007) of treatment. Also, correct prediction of persons who are unlikely to have TB is important as well to limit the expense and potential toxicity of empirical therapy. This requires a novel method for rapid diagnosis of tuberculosis (TB) which is urgently needed to complement smear microscopy, which has low sensitivity, and culture which is slower and requires specialized laboratory conditions not available in resource constrained settings.

While the occurrence of TB presenting in HIV epidemic setting has been recognized, the clinical features of TB manifesting itself in the absence of radiographic abnormalities have not been closely examined and thus are not well known. Also, there are no clear clinical impressions and other demographic factors suggesting an increasing incidence of smear negative TB and the lack of objective data describing pulmonary TB with a normal/abnormal Chest X-ray.

In the absence of clear ways of identifying patients with active TB who have negative sputum smear results, case detection rate would decrease and the transmission of the TB infections would increase in the community(Odusanya and Babafemi J, 2004). In addition, with no proper identification of persons who have TB, it would be difficult for appropriate isolation procedures to take place and to provide a basis for early institution of therapy. Therefore, correct prediction

of persons who are unlikely to have TB is important to limit the expense and potential toxicity of empiric therapy.

1.2 Justification

Many countries adopted the WHO guidelines and included an algorithm for the diagnosis of smear-negative pulmonary tuberculosis in their national guidelines. In this guideline, the examination of up to nine sputum smears is recommended before the diagnosis of smear-negative tuberculosis is reached in some of the countries (WHO, 2003). Clinical peer review, or discussion of the case by a clinical team, is supposed to be used to establish the diagnosis of smear-negative pulmonary tuberculosis case under routine programme conditions in some countries.

Given that most of the health facilities in Uganda are inadequately equipped, and TB sputum smear not being very reliable, it is important to identify the clinical, demographic, and radiographic predictors for smear-negative TB to aid clinicians in predicting the likelihood of TB in persons with negative sputum smear results, especially in lower health facilities. In addition, it is important to identify difficulties and ways to improve the diagnosis of smear-negative tuberculosis, especially in settings with high rates of HIV infection, and propose changes to national and international tuberculosis control policies.

In addition, instituting a more sensitive diagnostic tool will prevent the unnecessary cost of treating individuals who do not have TB or leaving out TB cases untreated and at the same time it will prevent the further spread of TB and deaths associated with it (Hargreaves Kadzakumanja and Whitty etal, 2001). This emphasizes the need of simple and effective tools which can be

used to predict TB cases in health facilities and the need for further research in order to identify a better diagnostic tool for diagnosis of TB.

1.3 Scope

The framework relates factors that could independently predict the sputum smear negative tuberculosis and the likely consequences. They included socio-demographic, clinical, human and laboratory factors. The scope of this study involved all the predictors except laboratory associated factors and the consequences.

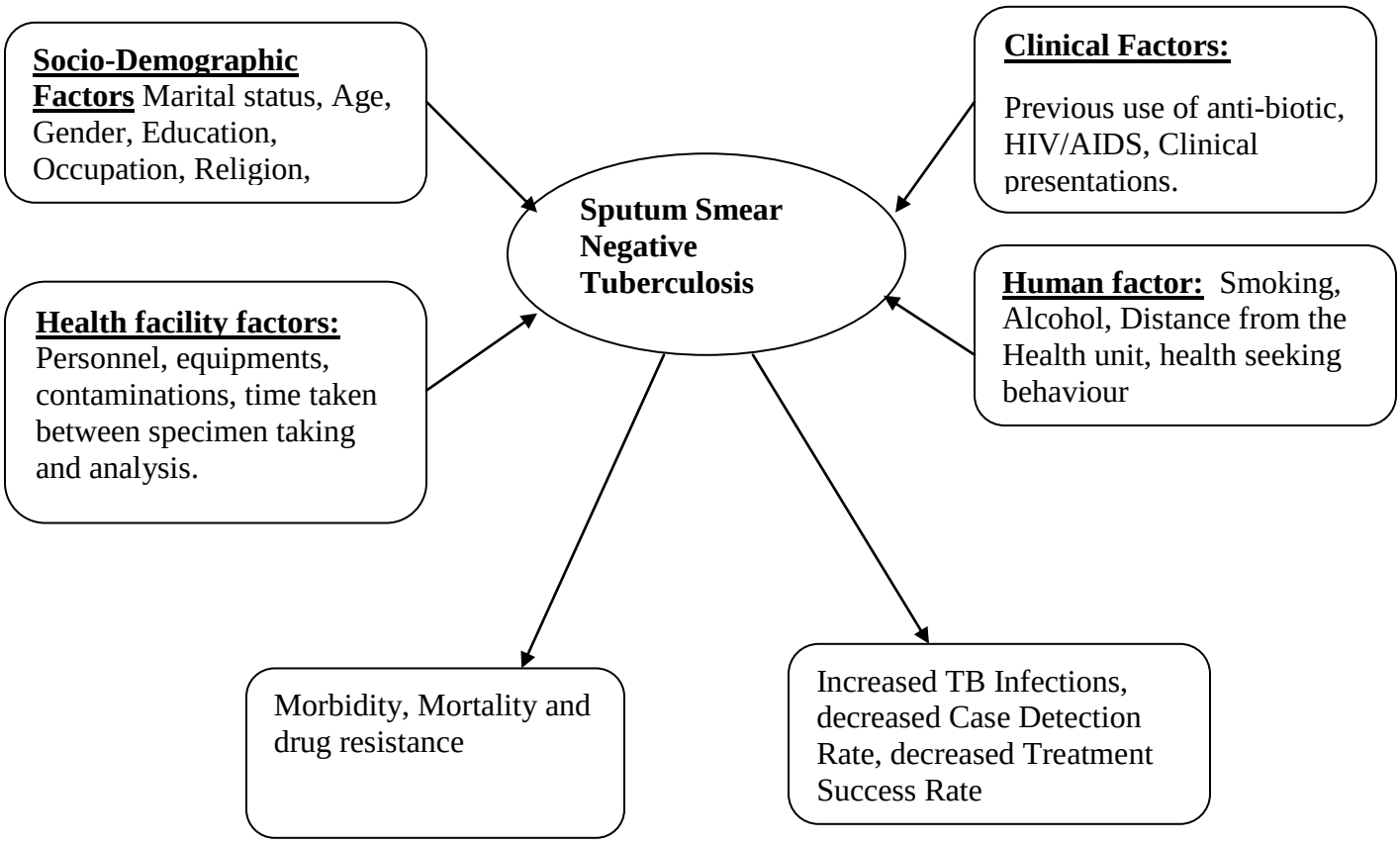

Figure 1: Conceptual framework showing independent factors possibly associated with sputum smear negative Tuberculosis

1.4 Research Question

i. What is the prevalence of smear negative Tuberculosis among adult TB patients from Kawempe-Kampala attending CWRU-MU Research collaboration clinic between January 2002 to December 2012?

ii. What are the factors associated with smear negative Tuberculosis among adult TB patients from Kawempe-Kampala attending CWRU-MU Research collaboration clinic between January 2002 to December 2012?

1.5 Hypothesis

H_0: There is no association between HIV/AIDS infection and smear negative tuberculosis among adult TB patients from Kawempe-Kampala attending CWRU-MU Research collaboration clinic between January 2002 to December 2012.

H_A: There is association between HIV/AIDS infection and sputum smear negative Tuberculosis among adult TB patients from Kawempe-Kampala attending CWRU-MU Research collaboration clinic between January 2002 to December 2012.

1.6 Objective

1.6.1 Purpose of the study

The main aim of the study was to determine the prevalence and factors associated with sputum smear-negative Tuberculosis among adult TB patients from Kawempe-Kmapala attending CWRU-MU Research collaboration clinic between January 2002 to December 2012.

1.6.2 Specific objectives

i. To determine the prevalence of smear negative Tuberculosis among adult TB patients from Kawempe-Kampala attending CWRU-MU Research collaboration clinic between January 2002 to December 2012?

ii. To determine factors associated with TB smear negative result among adult TB patients from Kawempe-Kampala attending CWRU-MU Research collaboration clinic between January 2002 to December 2012?

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

Microscopic examination of sputum smears for acid-fast bacilli is used widely throughout the world as a diagnostic test in people who are suspected of having pulmonary tuberculosis. The finding of acid-fast bacilli in sputum establishes a presumptive diagnosis of tuberculosis and indicates that the patient is capable of transmitting the infection.

2.1 Prevalence of Sputum Smear negative

There are millions of new smear-negative TB cases each year worldwide (WHO, 2012), but still the proposed strategies for diagnosis are still unclear, especially at primary health care setting that rely heavily on clinical judgment, which may be appropriate for settings with well trained medical personnel. But where the decisions rely on non-specialized health workers, it may leads to a substantial increase in number of under diagnosis TB. In addition, little attention has been given to smear-negative TB compared to smear positive cases, which is considered the most effective strategy to curb the tuberculosis epidemic making the diagnosing smear-negative TB not being considered a public health priority for tuberculosis programs. Yet it is believed that the consequences of under diagnosis of tuberculosis as a result of high prevalence of smear-negative tuberculosis cases are responsible for approximately 20% of tuberculosis transmission (Behr et al. 1999).

Over dependency on microscopy as a base line tool for detecting tuberculosis, especially in resource constrained countries has been a challenge due to its low sensitivity and inaccuracy in operation. Although there are other contributing factors, this has led to increase in the sputum smear negative tuberculosis.

The WHO DOTS strategy for tuberculosis control was used to diagnose and treat more than 21 million patients with tuberculosis between 1995 and 2004 (WHO, 2006). This strategy recommends identification of infectious tuberculosis cases by microscopic examination of sputum smears to identify acid-fast bacilli. However, the HIV epidemic has led to huge rises in incidence of tuberculosis in the worst affected countries, with disproportionate increases in smear-negative pulmonary tuberculosis (Colebunders R, et al, 2000). Therefore the challenges mentioned above, which is associated with microscopy and its use as a base line tool for detecting tuberculosis, would mean increase in smear negative tuberculosis.

The strong association between HIV infection and tuberculosis in sub-Saharan Africa has led to an upsurge of tuberculosis in many countries in the region. Countries with good tuberculosis surveillance and notification systems, such as Burundi, Malawi, Uganda, the United Republic of Tanzania and Zambia, have seen large increases in notification rates over the last 10 years, particularly for patients notified as suffering from sputum-smear-negative pulmonary tuberculosis (WHO, 1996). However, some of these notifications are based on microscopy meaning those with clinical presentation suggesting TB but with smear negative sputum are left out.

Direct Ziehl-Neelsen (ZN) sputum smear microscopy for the detection of acid fast bacilli (AFB) remains the most important diagnostic test for tuberculosis (TB) in high burden countries (WHO, 2008). Although rapid, specific, and appropriate for laboratories with minimal infrastructure, sputum microscopy failed to detect 56% of the estimated global burden of new TB cases in 2006 (Keeler E; Perkins MD Small P; Hanson C; Reed S; et al, 2006). The proportion of missed cases may be higher among HIV infected patients, who are often, smear negative despite multiple smears. In Uganda, a total of 49,009 TB cases were notified and 25,448 (52%) of them were smear positive TB cases, while 12,824 were Sputum smear negative (NTLP data, 2011). In addition, data on factors associated with increased risk of smear negative TB is still scarce.

In a study of a community in a township in Cape Town, South Africa, the antenatal HIV seroprevalence rate was 30%, and the annual TB notification rate increased to 11500 cases per 100,000 population—almost 200-fold higher than TB rates in The Netherlands (WHO, 2011). This has been associated with a major and disproportionate increase in the rate of smear-negative disease among HIV-infected individuals. Moreover, the prevalence of undiagnosed TB (a key determinant of transmission) among HIV-infected patients in this community is very high, and disease duration before diagnosis is prolonged (Wood R; Middelkoop K; Myer L; et al, 2007). In view of these observations, transmission attributable to smear-negative pulmonary TB cases at the community level may be a very important element of public health concern.

Tuberculosis infected children are usually sputum smear negative and they normally do not contribute to the immediate spread of the disease (Lawn, 2008). However, TB infected children form a pool of infection from which a significant number of future adult cases arise. For these

reasons, children in contact with adult patients are at highest risk of getting infected, and hence contact screening is important for early detection of transmission of infection (Altet Alcaide Plans P, 1996). This is of great value not only to detect infected individuals but for the community as a whole, because a child with tuberculosis represents recent and ongoing transmission of infection within the community.

The uses of other drugs either anti-biotic or antiretroviral also have contributed to the increase of sputum smear negative test results. The high burden of TB in these kinds of clinical settings presents a great challenge with regard to morbidity, mortality, and risk of TB transmission (Lawn; Bekker; Middelkoop; et al, 2006). A study in Malawi, including follow-up data (7 years), reported that patients with smear-negative pulmonary tuberculosis had a significantly higher risk of death than patients with smear-positive tuberculosis, with a hazard ratio of 2·2 (LawnSD, 2008). Most people start using anti-biotic for treatment of cough which sometimes turns out to be TB, hence increasing the possibility of sputum testing negative.

2.3 Factors associated with sputum smears negative test result

In most resource-constrained settings, access to health services and DOTS is restricted and services reach only a fraction of the population. If the availability of these services were increased, it is expected that a much higher frequency of TB would be seen. Negative smears could also be the result of poor quality smear microscopy from inadequate sputum collection, storage, and staining, reading errors, or poor laboratory services. In children, the diagnosis of pulmonary tuberculosis is especially difficult because the disease is paucibacillary and collection of sufficient sputum for smear microscopy and culture is difficult (Chintu Mwaba, 2005).

Several studies have identified factors predictive of tuberculosis in AFB-negative HIV-infected patients (Le Minor; Germani; Chartier L; et al, 2008). However, few have focused on clinical and radiological characteristics associated with AFB negativity in co-infected patients (Mwandumba;Bertel Squire; et al, 2008) and few have included in-depth search for etiological agents of respiratory symptoms by means of bronchio-alveolar lavage (BAL). Furthermore, the epidemiological context differs from country to country, and predictive factors can also vary which need to be studied depending on the country.

2.4 Sputum and blood cultures

Sputum culture is the gold standard for the diagnosis of tuberculosis and its use could substantially reduce cases of wrong diagnosis of TB which in turn translates into high rates of morbidity and mortality among TB patients. However, in resource-poor settings, culture is recommended selectively and is mainly used for surveillance of drug sensitivity to confirm treatment failure and relapse, and in pulmonary tuberculosis patients with repeated negative smear results (WHO, 2003). As a result many resource-poor setting depend on sputum smear microscopy and with its challenges, most suspected TB cases are left undetected. For example, in a study of HIV-positive tuberculosis patients in Khayelitsha, South Africa, 49% of patients on tuberculosis treatment had negative smears on direct microscopy but their sputum cultures were positive.

In addition, Mycobacterium are slow-growing organisms but culture takes 6–8 weeks and needs reasonably sophisticated facilities and technical expertise(WHO, 2006b). Thus its usefulness is restricted, especially in resource-constrained settings that lack reasonably sophisticated facilities and technical expertise.

2.5 Chest radiography

Although the classical radiographic hallmarks of PTB are cavitation, apical distribution, bilateral distribution, pulmonary fibrosis, shrinkage and calcification, no pattern is absolutely diagnostic of tuberculosis. Patients with HIV infection may have atypical radiographic findings such as infiltrates without cavitations (involving particularly the lower lobes) and hilar lymphadenopathy. The radiographic presentation is related to the CD4 lymphocyte count. A study in Canada (GreenbergSD., 1994; Kelper, 1995) found that the mean CD4 lymphocyte count in HIV positive PTB patients was 323 cells per patient when the chest radiograph was "typical" and 69 cells per patient when it was "atypical". Similar findings have been reported from Cote d'Ivoire (Abouya, 1995) and South Africa. It is important therefore, to determine the sputum smear result (negative or positive) which is associated with radiographic result suggestive of Tuberculosis in the diagnosis.

Interpretation of chest X-rays of individuals suspected to have PTB is difficult. In the pre-HIV era, there was considerable inter- and intra-observer variation in chest X-ray interpretation by radiologists and chest physicians. In sub-Saharan Africa, the problem is compounded because there are few trained radiologists or chest physicians, and in most district hospitals chest X-rays are interpreted by relatively inexperienced medical officers or paramedics. The nonspecific findings of pulmonary infiltrates, in the middle or lower lobes, in HIV positive PTB patients adds to the difficulties of correct radiographic diagnosis. It is now well recognized in industrialized countries (GreenbergSD., 1994) and countries in sub-Saharan Africa that the chest X-ray can appear normal in HIV-positive PTB patients. In one study in the USA, 44% of HIV-positive tuberculosis patients with negative sputum smears and positive cultures of M.

tuberculosis had a normal or minimally abnormal chest X-ray (GreenbergSD, 1994). In health facilities where mycobacterial cultures are not available, such patients will probably not be recognized as having PTB and will therefore not receive antituberculous treatment. In Cote d'Ivoire, 44% of patients diagnosed with HIV wasting syndrome had tuberculosis at autopsy, the diagnosis not having been considered previously.

In countries implementing DOTs strategy in detecting TB using microscopy like Uganda, coming out with clinical presentations associated with negative smear sputum but with chest X-ray suggestive of tuberculosis would help a lot in the diagnosis of tuberculosis.

CHAPTER THREE

METHODS

3.1 Study Design

The study was a retrospective analysis of records involving an inception cohort of Tuberculosis patients from Kawempe Community study who were screened from CWRU-MU treatment center, Mulago hospital from 2002 to 2012. The study designs were cross sectional and a nested case-control study (Nested in an existing cohort of TB patients, that is, cases emerged from a well-defined source population or cohort and the controls were sampled from that same population). The main difference between a case-control and a nested case-control study is that in the case-control, the cases and controls are sampled from a source population with unknown size, whereas the nested case-control is 'nested' in an existing predefined source population (cohort) with known sample size.

3.2 Study Setting

The study was conducted at Case Western Reserve University – Makerere University (CWRU-MU) treatment center based at Mulago hospital (outpatient clinic). Case Western Reserve University – Makerere University Research Collaboration (CWRU-MU) is a joint collaboration research project between the two universities.

CWRU is based in Cleveland, Ohio state in the United States of America while Mulago is the national referral hospital in Uganda located 2 kilometers north of Kampala city center. This research collaboration dates back to the late 1980's. Currently there are about eight studies which are either observational or clinical trials ongoing at the site, all on TB and among them is the Kawempe Community study which used for this study. The research collaboration thrives on

funding from the National Institutes of Health and Centres for Diseases Control and Prevention both based in the US.

CWRU-MU also works jointly with other organizations like National Tuberculosis and Leprosy Programme (NTLP) - Ministry of Health (MoH), Joint Clinical Research Centre (JCRC), and National Tuberculosis Reference Laboratory (NTBRL). The national tuberculosis laboratory conducts sputum smear and JCRC carries out both sputum smear and culture tests of specimen taken from CERU-MU treatment site. That is, the tests conducted in these laboratories are; sputum smear, culture, isolation and genotyping and drug sensitivity testing of the Mycobacterium bacilli.

Kawempe is located in the north of Kampala city and it is one of the divisions with high environmental pollution due to many factories and encroachment on wet land and has some of the worst health indicators in Kampala, including high cases of TB and HIV infection.
The Kawempe Community Health Study is an epidemiological study being conducted in Kawempe Division in Kampala and it uses the household contact design with embedded cross-sectional and cohort hybrid designs. During the study, approximately 150 index cases of TB were enrolled per year. With an average family size of 5, approximately 450 household contacts are enrolled each year.

To be enrolled, the suspected TB cases from the community clinics are referred to Case Western Reserve University – Makerere University Research Collaboration (CWRU-MU) treatment site where both sputum smear and culture tests are done and those who are culture positive were enrolled into the study. Once enrolled, index cases are started on a standard regimen of

antituberculous therapy, followed monthly during therapy, and then quarterly thereafter for a minimum of one year after completion of treatment. The outcomes of interest in the index cases include cure, treatment failure, relapse, defaulters, multidrug-resistant (MDR) TB and death. Meanwhile the contacts are followed for about 24 months for any TB infection.

3.3 Study Participants

3.3.1 Target population:

All the suspected TB cases who were 18 years and above were enrolled for both cross sectional and case control studies.

3.3.2 Accessible population

All the suspected TB cases from Kawempe Division who were 18 years and above who had been screened for TB from 1st January 2002 to 31st December 2012 at CWRU-MU treatment center, Mulago.

3.3.3 Study population

All the TB patients who were 18 years and above with culture positive test result and had been initiated in TB care and treatment from 1st January 2002 to 31st December 2012 at CWRU-MU treatment center.

3.4 Eligibility Criteria

3.4.1. Inclusion for prevalence study

All the TB patients, 18 years and above, who had their sputum test results and culture result recorded in their files.

3.4.2. Exclusion criteria for cross sectional study

TB patients who were not from Kawempe division were not included. Also those who had been on TB treatment before and were being screened for re-infection were left out.

3.4.4 Inclusion Criteria for Case Control study

In case control study, the cases were TB patients who had their sputum smear negative but their culture test result turned positive. And the controls were all TB patients with both the sputum and culture test results positive.

Therefore, TB patients who were 18 years and above who had Sputum smear and Culture test results from CWRU-MU laboratory from 1st January 2002 to December 2012 and had their files available with complete records.

3.4.5 Exclusion Criteria

TB Patients with either unconfirmed sputum smear or culture results, those outside Kawempe division and those who were in both case and control arms, were excluded from the study.

3.5 Sample Size Determination

Objective 1: Determine the prevalence of smear negative TB

Cross sectional study, the minimum sample size was calculated using Kish Leslie (1965) formula given by,

$$\mathbf{n} = \frac{Z_{\alpha}{}^{2} P(1-P)}{d^{2}}$$

Where $Z_{\alpha}{}^{2}$ is the standard normal value at the confidence level of 95% (1.96), P is the estimated prevalence of sputum smear negative in Uganda which is at 26%, according to the national tuberculosis and leprosy data 2011 and d is the precision of 5%.

Therefore;

n = $\frac{1.96^2 x 0.26 x 0.74}{0.05^2}$ = 296 is the minimum number required for cross sectional study.

Objective 2: Factors associated with sputum smear negative TB.

Using Fleiss formula with continuity correction factor for calculation of minimum sample size for case control study:

Where; $n = \frac{[Z_\alpha \sqrt{P(1-P)(1/q_1 + 1/q_2)} + Z_\beta \sqrt{P_1(1-P_1)1/q_1 + P_2(1-P_2)1/q_2}]^2}{(P_1 - P_2)^2}$

n = Sample size in the group.

p_1 = 44%, proportion of sputum smear-negative patients who are HIV-who are HIV/AIDS positive (GreenbergSD., 1994)

p_2 = 60%, proportion of sputum smear-positive TB patients who are HIV/AIDS positive (Robert L; Smith; Kenneth Yew; et al, 1994).

P = $q_1p_1+q_2p_2$, p_1 = (0.44x0.56) + (0.6x0.4) = 0.486

q_1= 1, proportion of cases, q_2 = 3, proportion of controls.

Z_β = standard normal value corresponding to the desired power 80% power (0.84).

Z_α= desired level of statistical significance at 95% confidence level (typically 1.96). It gives the minimum sample size of 324 participants.

Thus using a precision of 5% for the descriptive objective, we would require a total sample size of 296 but for the analytical objectives by using a power of 0.8 we would require a minimum

sample size of 324, about 81 cases and 243 controls to detect a minimum odds ratio of 1.3. We would take the larger sample size.

3.6 Sampling Procedure

3.6.1. For cross sectional study

The minimum sample size needed was 296 but the sampling frame had only 825 patients which were manageable and in order to increase precision of the study, all the TB patients in the sampling frame were included in the study.

3.6.2 For Case Control Study

The sampling frame for cases were the list of the TB patients who were sputum smear negative and culture positive. Meanwhile the list of all TB patients who were sputum smear positive and culture positive constituted the sample frame for the controls. The study unit was all the TB patients at CWRU_MU treatment center. In each year, all the cases were consecutively sampled and the corresponding controls in that particular year were randomly selected. For each case selected in a particular year, three samples were randomly selected.

3.7 Study Variables

3.7.1 Outcome Variable

For case control study was **Sputum smear test result**.

3.7.2 Independent Variables

1. **Demographic factors;** sex, age, education level, occupation, tribe and marital status.

2. **Clinical factors;** cough, temperature, night sweats, clinical presentations, chest X-ray result and HIV seropositivity.

3. **Social/Risk Behavour;** known exposure to TB, distance to the nearest clinic, prior use of drugs, drinking alcohol, tobacco use, and other co-morbidities associated with TB, such as diabetes, cancer, or chronic steroid use.

3.8 Data Collection

Data were collected using a pretested structured data extraction form. Six trained research assistants extracted quantitative data from the patients' files which were stored in the Case Western Makerere University collaboration office. We recorded standard risk factors for TB infection and disease including clinical, demographic and risk behaviors. The result of the chest radiograph was categorized as having normal or abnormal.

3.9 Data management

Data were extracted by research assistants from the patients' files using structured data extraction forms and edited for omissions or errors during recording right in the office where the data collection was taking place. The completed forms were transported to Makerere Clinical Epidemiology Unit. The data were then double checked and entered into Epidata version 3.1 and exported to Stata version 10, statistical software for analysis.

3.10 Data Analysis

Descriptive statistics were used to summarize the baseline categorical characteristics of the study participants in form of proportions and percentages. Continuous independent variables (such as age, distance from the nearest health facility) were summarized into mean, standard deviation, median and inter quartile range. Bivariate comparisons between study patients and control patients was performed using χ^2 and Fisher's Exact Test for categorical variables or the t test

test for continuous variables where appropriate. Potential predictor variables for smear-negative TB whose p-value was less than 0.20 was considered for multivariate models All tests of significance were two sided; $p \leq 0.05$ was considered statistically significant. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to measure the effect of predictor variables on the outcome variable.

Multiple logistic regression analysis was done using software Stata 10 to identify independent predictors for negative sputum smear mycobacterium tuberculosis. The backward (stepwise) selection method, using logistic regression, was used to choose the final model and those which were statistically significant remained in the model. To assess for confounding, the variables which were dropped out during multivariate analysis were brought back into the model one by one and the model was ran using enter method. The percentage change (effect measure) in odds ratios between the reduced model and full model that is greater than 15% showed that there was confounding. The interaction was tested using chunk test where -2log likelihood of reduced and full models were compared and significant chi-square means there was interaction.

3.11 Quality Control

To ensure quality, research assistants were trained for three days and about ten data extraction forms were pre-tested. There was double editing both at the extraction site and data entry. Lastly there was sampling of a few completed extraction forms which were cross-checked with data in the original patient files.

3.12 Ethical Considerations

Ethical approval was obtained from Makerere University Clinical Epidemiology Unit, and the School of Medicine Institutional Review Board. The consent was obtained from the Case

Western Reserve University-Makerere University study center. No participant's name appeared on the data extraction form to ensure confidentiality. Waiver of consent was asked from School of Medicine Institutional Review Board.

CHAPTER FOUR

RESULTS

4.0 Socio-demographic characteristic for Prevalence Study

A total of 825 patients were used for prevalence study. The age of the TB patients were not normally distributed and skewed to the right (Fig. 2) with median 27 (inter-quartile range =12), and the median temperature of patients was 37.3^{0}C (inter-quartile range =1.3) and the distance to the nearest health facility walked by the patients had median distance of 5 Km (inter-quartile range=5)

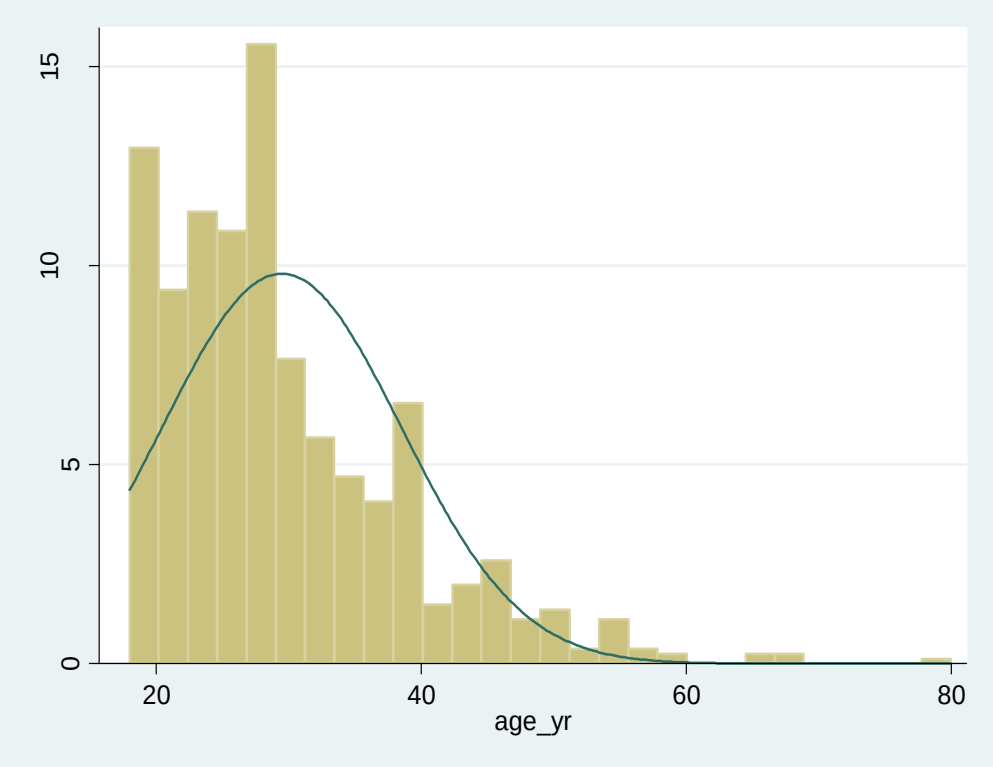

Figure 2: Age distribution of TB patients in Kawempe-Kampala 2012

A total of 825 patients were included in the study of, which 53.3% (433) were male and 46.7% (379) were female as in table 1 below. Also, 78.3% were 18-35 years old and those were marriage constitute the 47.3% (Table 1).

Table 1: Socio-demographic Characteristics of TB patients in the Prevalence Study in Kawempe-Kampala, 2013

Variable	Frequency	Percent
Gender (812)		
Male	433	53.3
Female	379	46.7
Education (807)		
None	51	6.3
Primary	318	39.4
Secondary	438	54.3
Employed (805)		
Yes	466	57.9
No	339	42.1
Marital Status (812)		
Never Married	241	29.7
Currently Married	384	47.3
Divorced, Separated and Widowed	187	23.0
Region of Origin (811)		
Central	518	63.9
West	137	16.9
East	72	8.9
North	40	4.9
Rwandese	25	3.1
Others[##]	19	2.3
Religion (811)		
Catholic	250	30.8
Anglican	241	29.7
Muslim	191	23.6
SDA	14	1.7
Others[**]	115	14.2
Smoking (812)		
Yes	170	20.9
No	642	79.1
Drinking Alcohol (712)		
Yes	183	22.5
No	629	77.5
HIV Status (825)		
Yes	226	27.4
No	597	72.4
Unknown	2	0.2

[##] *Include Kenyan, Tanzanian and South Sudanese, ** Born again, Baptish*

4.1 Prevalence of smear-negative TB

The overall prevalence of negative sputum smears among TB patients in Kawempe was 9.1% (95% CI, 0.071-0.111) and among HIV positive patients was 15.5% (95% CI, 0.108-0.202) as shown in the table 2 below.

Table 2: Prevalence of smear-negative TB in Kawempe-Kampala Uganda, 2013

Variable		Count	Prevalence (%)	95% CI (%)
Overall (N=825)		75	9.1	7.1-11.1
Age[¥]	18-35 (n=646)	57	8.8	6.6-11.0
	36<= Years (n=174)	18	10.3	5.8-14.8
Gender				
	Male (n=433)	39	9.0	6.3-11.7
	Female (n=379)	33	8.7	5.9-11.6
Education				
	None (n=51)	6	11.8	2.8-20.7
	Primary (n=318)	28	8.8	5.7-11.9
	Secondary (n=438)	37	8.4	5.8-11.1
Marital Status				
	Never Married (n=240)	23	9.6	5.8-13.3
	Currently Married (n=385)	23	6.0	3.6-8.4
	Divorced, Separated and Widowed (187)	26	14	8.9-18.9
Employed				
	Yes (466)	43	9.2	6.6-11.9
	No (339)	29	8.6	5.6-11.5
Smoking				
	Yes (n=170)	17	10	5.5-14.5
	No (n=642)	55	8.5	6.5-10.7
Alcohol				
	Yes (183)	17	8.7	4.6-12.9
	No (628)	56	8.9	6.7-11.2
HIV Status				
	Positive (226)	35	15.5	10.8-20.2
	Negative (597)	39	6.5	4.5-8.5
Anti Biotic use				
	Yes (n=441)	48	10.9	7.9-13.8
	No (337)	15	4.5	2.2-6.7

[¥] *Based on WHO classification (Youth 35 below and adult 36+)*

4.2 The Case Control Study

A total of 81 cases and 243 controls were sampled and included in the study to assess the factors associated with sputum smears negative tuberculosis (case control study).

4.2.1 Study Profile

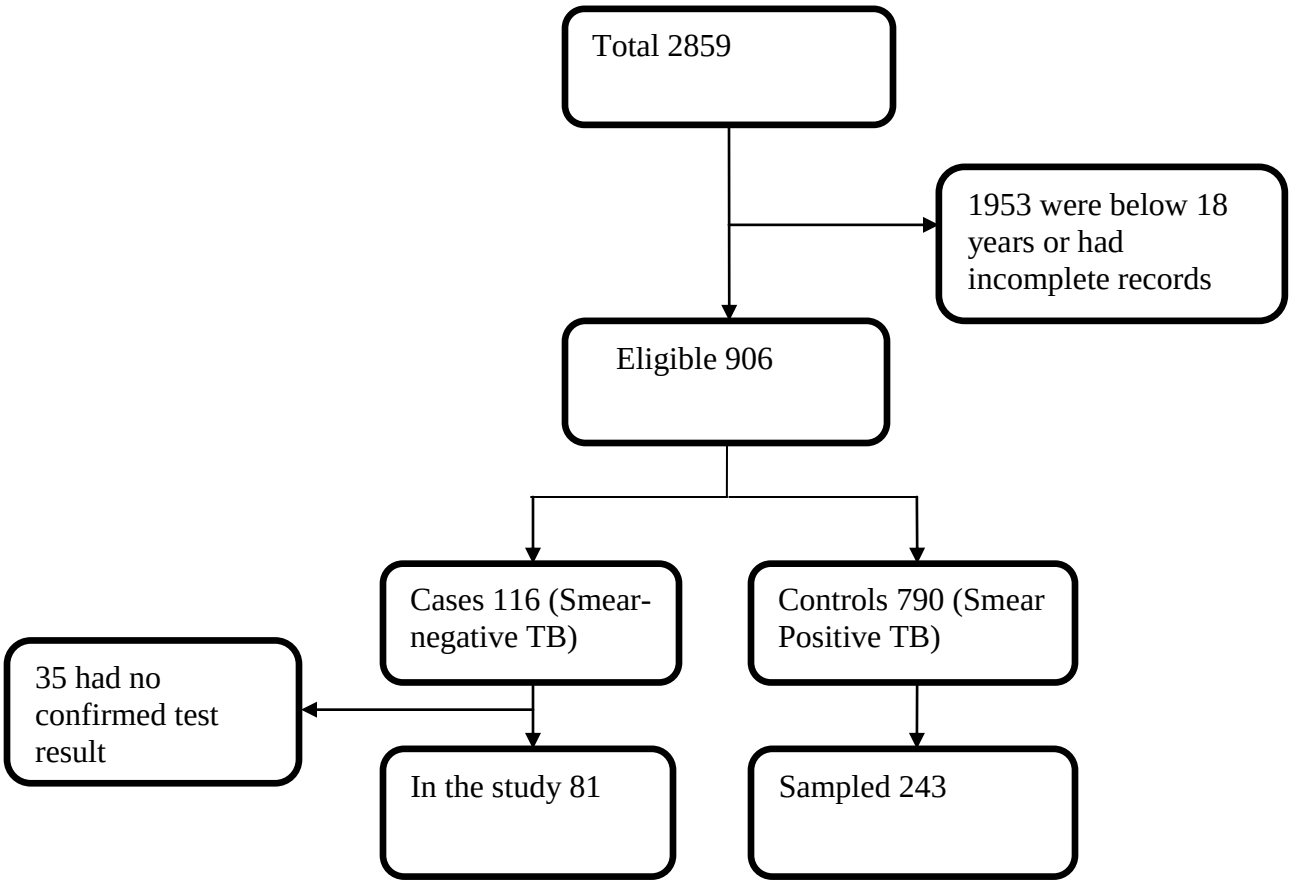

Figure 3: The study profile for case control study in Kawempe-Kampala, Uganda, 2013

4.2.2 Socio-demographic characteristics TB patients for case control study

There is no significant difference in age between the cases and the control groups since the p-value is greater than 0.05 (Table 3).

Table 3: Assessing for differences in age, temperature and distance to nearest unit for TB patients in Kawempe-Kampala, 2013 (t-test)

Variable	Case	Control	P-value
Age			
Median	29	27	0.64
Maximum	55	67	
minimum	18	18	
Temperature[€]			
Mean	37.2	37.3	0.14
Standard deviation	0.89	0.98	
Distance to the nearest health Unit[€]			
Mean	4.91	5.52	0.15
Standard deviation	3.17	3.19	

[€] *Normally distributed*

In table 4 below, 72.2% of the cases are 18-35 years compared to 76.7% in the control. Also 51.9% of the male were in case group and 54.8% were controls.

Table 4: Socio-Demographic Characteristics of both cases and controls TB patients in Kawempe-Kampala, 2013

Variable	Cases n (%)	Control n (%)
Gender		
Male	41 (51.9)	131 (54.8)
Female	38 (48.1)	108 (45.2)
Age		
=<35	57 (72.2)	184 (76.7)
>35	22 (27.8)	56 (23.3)
Education		
None	7 (8.9)	18 (7.6)
Primary	31(39.2)	94 (39.5)
Secondary	41 (51.9)	126 (52.9)
Employed		
Yes	42 (53.2)	94 (39.8)
No	37 (46.8)	142 (60.2)
Marital Status		
Not in marriage	42 (53.2)	108 (45)
Currently Married	37 (46.8)	132 (55)
Tribe		
Bantu	70 (88.6)	223 (94.1)
Non Bantu	9 (11.4)	12 (5.9)
Religion		
Catholic	23 (29.1)	88 (36.8)
Anglican	29 (36.7)	65 (27.2)
Muslim	17 (21.5)	53 (22.2)
SDA	2(2.5)	5 (2.1)
Others	8 (10.1)	28 (11.7)

Up to 68.8% of the cases move within 5Km radius to the nearest health facility compared to 53.3% controls (Table 5 below).

Table 5: Community/Human Characteristics of both cases and controls Kawempe-Kampala, Uganda, 2013

Variable	Cases n (%)	Control n (%)
[γ]Nearest Distance to health unit		
0-5 Km	53 (68.8)	123 (53.3)
6 and Above	24 (31.2)	108 (46.7)
Somebody ever smoked		
Yes	19 (24.1)	53 (22.2)
No	60 (75.9)	186 (77.8)
Currently Smoking		
Yes	5 (23.8)	10 (18.5)
No	16 (76.2))	44 (81.5)
Ever taken Alcohol		
Yes	14 (17.7)	43 (17.9)
No	65 (82.3)	197 (82.1)
Drinking Now		
Yes	14 (17.9)	41 (17.6)
No	64 (82.1)	192 (82.4)
Freq. of drinking		
Less than 1 day in a week	3(23.1)	10 (27.8)
2-3 days per week	4 (30.8)	17 (47.2)
4-6 days per week	3 (23.1)	4(11.1)
Daily	3(23.0)	5 (13.9)
Close Contact		
Yes	10(13.0)	36 (15.2)
No	40 (52.0)	120 (50.6)
Unknown	27 (35.0)	81 (34.2)

[γ] *Recommended distance by MOH to the nearest health unit should be within radius of 5Km*

The proportion of cases that previously used anti-biotic (71.1%) is higher than for controls (54.6%) and 45.7 % of the cases were HIV positive compared to 24.2% controls (Table 6 below).

Table 6: Clinical Characteristic of both cases and controls TB patients in Kawempe-Kampala, Uganda, 2013

Variable	Cases n (%)	Control n (%)
Previous use of Anti-biotic before test		
Yes	54 (71.1)	125 (54.6)
No	22 (28.9)	104 (45.4)
Previous ARV Use before the test		
Yes	1 (1.8)	3 (1.6)
No	55 (98.2)	188 (98.4)
HIV Status		
Positive	37 (45.7)	58 (23.9)
Negative	44 (54.2)	185 (76.1)
X-ray		
Normal	43 (54.4)	85 (35.9)
Abnormal	36 (45.6)	152 (64.1)
Duration between specimen collection and analysis		
One or less day	26 (32.1)	117(49.0)
More than one day	55 (67.9)	122 (51.0)

In table 7 below, about 81% (64) had fever, almost 60% (47) were sweating at night and all of them (100%) had cough.

Table 7: Clinical Presentation of both cases and controls TB patients in Kawempe-Kampala, Uganda, 2013

Variable	Cases n (%)	Control n (%)
Fever		
Yes	64 (81.0)	189 (78.8)
No	15 (19.0)	51 (21.2)
Sweat		
Yes	47 (59.5)	149 (62.1)
No	32 (40.5)	91 (37.9)
Cough		
Yes	79 (100.0)	238 (99.2)
No	0 (0.00)	2 (0.8)
Cancer		
Yes	1 (1.27)	1 (0.4)
No	78 (98.73)	238 (99.6)

4.3 Bivariate Analysis

Here we are looking for independent association between the dependent variable and a predictor variable. From the table 8 below, there is no variable which is independently associated with the dependent variable.

Table 8: Association between social demographic factors and sputum smears test results among TB patients in Kawempe-Kampala, Uganda, 2013

Variable	Cases n (%)	Control n (%)	ORs	95% CI	P-value
Gender					
Male	41 (51.9)	131 (54.8)	1.12	0.652-1.871	0.652
Female	38 (48.1)	108 (45.2)			
Age**					
=<35	57 (72.2)	184 (76.7)	1.27	.713-2.255	0.419
>35	22 (27.8)	56 (23.3)			
Education					
Primary	31(39.2)	94 (39.5)			
Secondary	41 (51.9)	126 (52.9)	0.97	0.581-1.609	0.897
Employed					
Yes	42 (53.2)	94 (39.8)	0.751	.449-1.255	0.275
No	37 (46.8)	142 (60.2)			
Marital Status					
Not in marriage	42 (53.2)	108 (45)			
Currently Married	37 (46.8)	132 (55)	0.72	0.433-1.200	0.208
Tribe					
Bantu	70 (88.6)	223 (94.1)			
Non Bantu	9 (11.4)	12 (5.9)	1.59	.577-4.400	0.369
Religion					
Catholic	23 (29.1)	88 (36.8)			
Anglican	29 (36.7)	65 (27.2)	1.71	.905-3.219	**0.098**
Muslim	17 (21.5)	53 (22.2)	1.23	.601-2.505	0.574
SDA	2(2.5)	5 (2.1)	1.53	.279-8.401	0.624
Others	8 (10.1)	28 (11.7)	1.09	.440-2.716	0.848

*** based on WHO classification (18-35 years are youths)*

Up to 68.8% of the cases walk within the radius of 0-5Km to the nearest health facility compared controls which is 53.3% (Table 9)

Table 9: Association between Human and Community factors and sputum smears test results among TB patients in Kawempe-Kampala, Uganda, 2013

Variable	Cases n (%)	Control n (%)	ORs	95% CI	P-value
Nearest Dist.					
0-5 Km	53 (68.8)	123 (53.3)			
6 and Above	24 (31.2)	108 (46.7)	0.52	0.521-1.054	0.21
Ever Smoked					
Yes	19 (24.1)	53 (22.2)	0.106	-.494-.705	0.730
No	60 (75.9)	186 (77.8)			
Currently Smoking					
Yes	5 (23.8)	10 (18.5)	0.318	-.898-1.534	0.608
No	16 (76.2))	44 (81.5)			
Ever taken Alcohol					
Yes	14 (17.7)	43 (17.9)	0.987	.507-1.919	0.969
No	65 (82.3)	197 (82.1)			
Drinking Now					
Yes	14 (17.9)	41 (17.6)	1.02	0.524-2.001	0.94
No	64 (82.1)	192 (82.4)			
Freq. of drinking					
Less than 1 day in a week	3(23.1)	10 (27.8)			
2-3 days per week	4 (30.8)	17 (47.2)	0.78	.145-4.244	0.778
4-6 days per week	3 (23.1)	4(11.1)	2.5	.346-18.039	0.363
Daily	3(23.0)	5 (13.9)	2.0	.291-13.738	0.481
Close Contact					
Yes	10(13.0)	36 (15.2)			
No	40 (52.0)	120 (50.6)	0.83	.379-1.830	.650
Unknown	27 (35.0)	81 (34.2)	1	0569-1.757	1.00

Those who previously used the anti biotic (OR=2.02, 95% CI=1.155-3.541), HIV positive TB patients (OR=2.6, 95% CI= 1.558-4.463) and Duration between (OR =2.03, 95% CI=1.193-3.450) are independently associated with sputum smear result (Table 10).

Table 10: Association between Clinical factors and sputum smears test results among TB patients in Kawempe-Kampala, Uganda, 2013

Variable	Cases n (%)	Control n (%)	ORs	95% CI	P-value
Previous Anti-biotic use					
Yes	54 (71.1)	125 (54.6)	2.02	1.155-3.541	**0.014**
No	22 (28.9)	104 (45.4)			
Previous ARV Use					
Yes	1 (1.8)	3 (1.6)	1.14	.116-11.173	0.911
No	55 (98.2)	188 (98.4)			
HIV Status					
Positive	37 (45.7)	58 (23.9)	2.60	1.558-4.463	**0.001**
Negative	44 (54.2)	185 (76.1)			
X-ray					
Normal	43 (54.4)	85 (35.9)	0.47	.278-.785	**0.004**
Abnormal	36 (45.6)	152 (64.1)			
Duration between specimen collection and analysis					
One or less day	26 (32.1)	117(49.0)			
More than one day	55 (67.9)	122 (51.0)	2.03	1.193-3.450	**0.009**

There is no independent association between sputum smear negative with any clinical presentation (Table 11).

Table 11: Association between Clinical presentation and sputum smears test results among TB patients in Kawempe-Kampala, Uganda, 2013

Variable	Cases n (%)	Control n (%)	ORs	95% CI	P-value
Fever					
Yes	64 (81.0)	189 (78.8)	1.151	0606-2.187	0.667
No	15 (19.0)	51 (21.2)			
Sweat					
Yes	47 (59.5)	149 (62.1)	0.897	.534-1.508	0.682
No	32 (40.5)	91 (37.9)			
C ough					
Yes	79 (100.0)	238 (99.2)			0.414[##]
No	0 (0.00)	2 (0.8)			
Cancer					
Yes	1 (1.3)	1 (0.4)	1.2	.757-1.915	0.432
No	78 (98.7)	238 (99.6)			

[##] *Few observations in the cell, exact test done and odds ratio CI not obtained*

4.4 The multivariate Analysis

After bivariate analysis, independent factors that had p-value of less than 0.2 with sputum smear negative result were considered for multivariate analysis. Those known in the literature to be associated with sputum smears negative were also considered.

Table 12: Logistic Regression of Sputum Smears Result on Independent Factors in Kawempe-Kampala, Uganda, 2013

Variables	ORs	P-value	95% CI
HIV positive TB Patients	1.87	0.041	1.026-3.398
Previous use of Anti-biotic by TB patient	1.76	0.045	1.039-3.422
Normal X-ray result	0.54	0.040	0.302-0.972

All the interaction terms which were formed between the main predictor (HIV) and the other three variables were dropped when stepwise method was used to run the model. Checking for confounding using the variables which were dropped, none of them could cause a change of more than 15% meaning that there was no confounding.

CHAPTER FIVE

DISCUSSION

5.1 Prevalence of Smear Negative TB

The overall prevalence of sputum smears negative among TB patients was 9.1%. The result from this study showed a slightly lower prevalence of smear-negative TB when compared to the national level of 26.2% (2011 NTLP data). This could be explained by the nature of environment in which study was done which was close to an ideal situation, that is, well trained laboratory staff, well equipped and less contamination.

The low prevalence in this study could be explained by the nature of laboratory environment being used where this study was conducted. Here the laboratories had almost an ideal environment that is, well equipped. This may not be the case to the laboratories environment at the lower level health facilities, even district hospital in Uganda. Given that, in most low income countries, the diagnosis of Mycobacterium still relies on the search for Acid-Fast Bacilli (AFB) in sputum smears, which has sensitivity between 50 and 80% in well-equipped laboratories (Aber et al., 1980). In low-income countries (Uganda inclusive), poor access to high-quality microscopy services contributes to even lower rates of AFB detection among suspected TB patients.

From the study, smear-negative tuberculosis was found to be more common in older than younger patients, that is, higher among those who were above 35 years. This is in line with study done by Samb and others which found that smear-negative TB is common among older TB patients in a country with low prevalence of HIV infection (Samb et al., 1999). However,

according to Parry, countries with high HIV prevalence have an even age distribution, probably because HIV affects younger age-groups (Parry, 1993).

5.1.1 Prevalence of Smear Negative TB among HIV Positive Patients

The prevalence of sputum smear negative was found to be high among those who are HIV positive (15.5%). The results shows that smear negative TB were high among HIV patients and this is in agreement with other studies. For example, a study in Zambia done on over 100 patients with culture positive TB found that 24% of those who were HIV-seronegative had a negative sputum smear, compared with 43% of those who were HIV-seropositive (Elliott et al., 1993). Other studies showed that the proportion of cases of smear-negative pulmonary tuberculosis in HIV-positive tuberculosis patients ranged from 10% to 61% (Chintu and Mwaba, 2005). For example, in a study of HIV-positive tuberculosis patients in Khayelitsha, South Africa, 49% of patients on tuberculosis treatment had negative smears on direct microscopy but their sputum cultures were positive.

The apparent variation in the prevalence of negative sputum smear between this study and other studies including that from Uganda National Tuberculosis and Leprosy Program is because of differences in the study populations. Some studies were conducted among patients seen at specialist institution-based centers (e.g. MU-CWRU TB study center) who may be more or less likely to be smear-positive depending on the referral procedure. The level of immunosuppression among the HIV-positive patients in the various studies may also have differed. Less severely immunocompromised HIV-positive patients tend to have classic cavitary TB which is smear-positive (Desta et al., 2009)

5.1.2 Prevalence of Smear Negative TB among Antibiotic users

The study found that the smear-negative TB prevalence among those who used the antibiotic before the test was 10.9%. This is also in agreement with other findings that the use of other drugs especially anti-biotics or antiretroviral therapy also have contributed to the increase in sputum smear negative test results. For a smear to be positive, there must be at least 5000-10 000 acid-fast bacilli per mL sputum. However, antibiotic action could reduce these quantities to a level which may be a bit hard for the microscopy to detect. The high burden of TB in these kinds of clinical settings presents a great challenge with regard to morbidity, mortality, and risk of TB transmission (Lawn; Bekker; Middelkoop; et al, 2006)

It is important therefore that, the clinicians should not solely rely on the results of smear microscopy to initiate TB treatment. Although rapid, specific, and appropriate for laboratories with minimal infrastructure, sputum microscopy failed to detect 56% of the estimated global burden of new TB cases in 2006 (Keeler E; Perkins MD Small P; Hanson C; Reed S; et al, 2006). This clinical judgment is appropriate where other diagnostic tools are scarce but there is potential of under diagnosis of smear negative tuberculosis as the main diagnostic tool is smear microscopy in most of the area outside the capital Kampala since radiology services are scarce and not always operational.

5.2 Factors associated with Smear-Negative Tuberculosis

The study found that there was significant association between HIV/AIDS and sputum smear-negative result (OR = 1.87, p = 0.041) and That TB patients who were HIV/AIDS positive had

higher chances to have sputum smear-negative compared to those who were HIV negative. This effect measure (OR) is slightly lower than that found in Senegal which was 2.6 (Samb et al., 1999)

Also previous use of Antibiotic (like amoeyl, amoxicillin, arithromycin, azithroazithromcin, erythromycin and promethazine) by the suspected TB patient was found to have significant association (OR = 1.76, p = 0.045), with sputum smear-negative.

And Chest X-ray was found to be a very important tool in the diagnosis of smear-negative TB (OR =0.54, p = 0.040). However, in sub-Saharan Africa with limited microbiological services, the problem is compounded because there are few trained radiologists or chest physicians, and in most district hospitals chest X-rays are interpreted by relatively inexperienced medical officers or paramedics. For example, survey in Malawi showed that medical officers misdiagnosed a third of clinical vignettes, which described typical radiographic signs of tuberculosis (Nyirenda et al, 1999).

It is now well recognized that the chest X-ray can appear normal in HIV-positive TB patients as a study in sub-Saharan Africa revealed that tuberculosis patients with HIV infection are more likely to have atypical chest radiographic appearance and even normal appearance than tuberculosis patients without HIV infection (Banda et al, 2000). In areas of high HIV and tuberculosis prevalence, 75% of patients with smear-negative tuberculosis are likely to have atypical chest radiographic findings. Patients with smear-negative tuberculosis are less likely to have cavities on the chest radiograph (odds ratio 2·56) than patients with smear positive

tuberculosis(Tessema et al, 2001). In addition, smear-negative patients can also present with normal or only slightly abnormal chest radiographs.

This means that the accuracy of the radiograph in detecting AFB is much reduced in TB patients who are HIV positive and in those who have been taking antibiotics. According to Angeby and others, the accuracy of both microscopy and radiography is reduced by HIV, and so assessment of diagnostic approaches with existing methods and continuing research into new diagnostics are necessary (Angeby et al., 2004).

5.2.1 Demographic Characteristic

All the socio-demographic factors (Age, education, religion etc) were not independently associated with smear-negative TB at bivariate level. This is in agreement with other studies, for example, in an area with low prevalence of HIV infection and high prevalence of tuberculosis, one study based in Senegal found no demographic characteristics differentiating smear-negative from smear-positive tuberculosis other than the absence of cough (Hargreaves etal, 2001)..

5.2.2 Human and Community Factors Associated

Smoking, alcohol and distance from nearest health facility were not independently associated with smear-negative TB at bivariate level. However, the duration of more than one day between the collection of sputum and conducting laboratory test was independently associated with the smear-negative TB, that is for those which take more than one day the chances of getting smear-negative is about two times more compared to those who take one day or less.

At the community level suspected TB patients are sometimes given specimen bottle to collect the sputum from home and in this case we expect that a much higher frequency of smear-negative would be seen due to contaminations as a result of poor storage or specimen taking. Negative smears could also be the result of poor quality smear microscopy from inadequate sputum collection, storage, and staining, reading errors, or poor laboratory, which sometimes result in contaminations.

In low resourced countries, over-worked TB control programmes, laboratories cannot cope with the influx of diagnostic and follow-up smear examinations, and smears may not be done at all. For example, in Botswana in 1992, 48% of patients reported with pulmonary tuberculosis had no smear examinations performed (De Cock and Wilkinson, 1995). Alternatively, the sputum specimens collected may be inadequate in quality or number. Ipuge et al. (Ipuge et al., 1996) found that 83.4% of smear-positive cases were detected on the first specimen, 12.2% on the second, and 4.4% on the third, by Ziehl-Neelsen staining under routine programme conditions in Tanzania.

5.2.3 Clinical Presentation

The presentation of fever, cough, night sweats in the suspected TB patient were not independently associated with smear-negative TB at bivariate level. It is possible that the clinical presentations of a suspected TB patient do not play a significant role in events regarding sputum smear-negative test result.

A study in Tanzania and Burundi identified four clinical criteria for diagnosis of smear-negative tuberculosis (Samb et al., 1997): presence of cough for longer than 21 days (OR 5·43, 95% CI=

1·95–15·1); presence of chest pain for longer than 15 days (OR=1·98, 95% CI0·77–5·12); absence of expectoration (OR= 0·42, 95% CI=0·15–1·18]). Much as their odds ratios showed some relation, some like chest pain and absence of expectoration were not significantly associated with the smear-negative.

5.2.4 Clinical Factors

The history of previous use of antibiotic (like amoeyl, amoxicillin, arithromycin, azithroazithromcin, erythromycin and promethazine) by suspected TB patients, HIV/AIDS positive patients and the chest X-ray were independently associated with sputum smear-negative TB. As can be seen from the result, the normal chest X-ray seem to be significantly helpful in the diagnosis of sputum smears-negative result and Chest X-ray plays a crucial role in the diagnosis of smear-negative TB. Also World Health Organization (WHO) recommends the use of chest radiography in diagnosis of TB suspects with negative smear microscopy results (WHO, 2007); however, this is not available everywhere in Uganda and where the DOTs strategy is being implemented using exclusively microscopy, the situation could be made worse. Although microscopy for the detection of AFB is rapid, low cost, and detects the most infectious cases of tuberculosis, it needs maintenance of equipment, consistent supply of reagents, and proper training in interpretation of the slides, which may be lacking especially where DOTs strategy is being implemented. In the absence of other diagnostic tools, the diagnosis of smear-negative is often based on clinical grounds. But clinicians often are reluctant to treat smear-negative TB because they lack certainty of the diagnosis (Basinga P, Moreira, 2007). This may lead to under-diagnosis of smear-negative TB.

In addition, in sub-Saharan Africa with limited microbiological services, the problem is compounded because there are few trained radiologists or chest physicians, and in most district hospitals chest X-rays are interpreted by relatively inexperienced medical officers or paramedics. Therefore with good routine reporting systems, the national tuberculosis program of countries such as Malawi and the United Republic of Tanzania have reported a larger increase in new cases of smear negative than of smear-positive TB in the last 10 years (Graf, 1994). This could be the case in Uganda where routine reporting come from poorly equipped laboratories and staff.

This finding also suggests that potentially under-diagnosis of TB exists in Uganda, especially in the rural settings where the uses of antibiotics are common without proper diagnosis. Most people start using anti-biotic for treatment of cough which sometimes turns out to be TB, hence increasing the possibility of sputum testing negative. Cough persisting for longer than 3 weeks warrants AFB microscopy, according to the current WHO guidance. However, one study, in an area of high HIV and tuberculosis prevalence, confirmed smear-negative tuberculosis in 35% of patients with cough unresponsive to antibiotics of only 1–3 weeks duration (Banda et al., 19980).

5.3 Strength of the study

Many studies done focused mainly the effect of co-morbidity on sputum smear result but this study has been able to investigate community factors which tend to influence sputum smear results too. Secondly, non response bias was minimized in this study with over 95% reporting on most variables in the data extraction tool meaning that selection bias was minimized.

5.4 Limitation of the study

The study had some limitations and one of them is that we were not able to compare what happened in CWRU-MU treatment sites with the general health care system in Uganda. First the result of this study may have been influenced by the nature of environment in which the study is being conducted. The study was conducted within a study in CWRU-MU treatment site, which has almost an ideal environment in terms of staff training, equipments and other resources necessary. This is not the case in most of the Government facilities, especially at community level where most of these are missing, hence making it a bit challenging in generalizing it to the general health care system in Uganda. However, CWRU-MU treatment site is being housed in Mulago hospital and using most of the staff from Mulago and national TB laboratory, which are Government facilities. The system here we assumed to be almost the same with the lower health care delivery.

We were not able to measure the laboratory factors and also we could not generalize the laboratory factors where the study was done to the general laboratory factors in the Government facilities where majority of the population are served.

Selection bias may have occurred since the study was done in a place where not everybody deep in the community could have been informed about MU-CWRU treatment site or every clinic in Kawempe is aware that they were supposed to refer any suspected TB patient here. However, patients came from diverse backgrounds both from within the city and from far beyond indicating that the sample was more likely to be representative.

The studies performed were based on reference hospital populations, so our findings may not be generalisable to primary care settings. This is not of concern for microbiological methods and the clinical algorithm test, since they have been designed specifically, and can be applied in hospital settings. In contrast, generalisability to the primary care level is an important issue for the clinical prediction rule (CPR) which does not rely on sophisticated technology and could be used at that level.

The variables we used were pre-determined so we could not come with something outside we would like to measure. This has led to some missing data and using secondary data could not allow for the probing of the patients for clarification. The presence of soft copy has helped in cross checking for the missing data from the patients' forms.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1) The prevalence of smear-negative TB according to this study is lower than the national figure which is at 26%.
2) The prevalence of smear negative TB among the HIV positive TB patients was found to be higher compared to HIV negative TB patients but low if you compared with other countries like Zambia which is at around 43%.
3) The prevalence of smear-negative TB among those who had the history of previous use of antibiotic before the sputum smear is higher than in those who did not use the antibiotic but lower than world over estimate of 56% by WHO.
4) HIV/AIDS and TB co-infection is the major factor that is associated with sputum smear-negative results. This made HIV positive TB patients to be about two times more likely to have sputum smear negative result compared to HIV negative TB patients.
5) The suspected TB patients who used the antibiotic before the testing for TB are bout two times more likely to have sputum smear-negative result compared to those who have not used the antibiotic before. has helped in cross checking

6.2 Recommendations

1. The diagnosis of tuberculosis should start from the primary care level with the combination of Chest-X ray and possibly guided by the use of a Clinical prediction Rules.

2. It is highly recommended that balanced investments are done at least between human experts and machines in order to make the best use of all available diagnostic options.
3. Screening of HIV/AIDS and TB should be done concurrently.
4. Proper guidelines for the use of antibiotic in the treatment of TB related disease should be developed and disseminated down to the community level.

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APPENDICES

Appendix A*:* Data Extraction Form for the study on

PREVALENCE AND FACTORS ASSOCIATED WITH SPUTUM NEGATIVE SMEAR AMONG TUBERCULOSIS PATIENTS IN KAWEMPE DIVISION-KAMPALA.

Name of Research Assistant……

Participant's ID No……..

(Write in bold or tick where appropriate)

A. Socio-Demographic Characteristics

1. Age (*In complete years*)_____

2. Gender:
   1. Female 2. Male
3. Religion
   1. Catholic 2.Protestant 3.Muslim 4. Other (Specify)_____
4. Marital status
   1. Single 2.Married 3.Separated/Divorced/Widowed
5. Education level attained
   1. Never gone to school 2.Primary 3.Secondary 4.Tertiary
6. Occupation
   1. House wife 2.Farmer 3.Business person 4.Salaried employee
   5. Other (specify)_____
7. Tribe
   1. Bantu 2. Not Bantu 3. Unknown
8. Division of residence
   1. Kawempe 2. Outside Kawempe

B. Social/Drug-Related Factors

9. Distance from the nearest health facility

1. Less than 1Km 2. 1-2Km 3. 3-4Km 4. 4-5Km 5. More than 5Km

10. Alcohol use

0. No 1. Yes

10b. if yes, frequency of alcohol use

1. 1 Day in a week 2. 2-3 Days/week 3. 4-6 Days/week 4. Daily

11. Smoking

0. No 1. Yes

11b. If yes, average number of cigarettes/day? _____

12. Use of other habit forming drugs

0. No 1. Yes

12b. if yes, specify the type_____

13. Use of Herbal(Local) medicines

0. No 1. Yes

14. Is the person on HAART

0. No 1. Yes

14b. if yes, for how long _____Month(s)

15. What other antibiotics has he/she been using in last 2 weeks

_____

_____

C. Medical History

16. Any close contact with TB patient before?

0. No 1.Yes 2. Unknown

16b. If yes, for how long _____months

17. Currently live with a contact with TB?

0. No 1. Yes 2. Unknown

Has he/she been diagnosed with any of the following conditions?

18. HIV 0= No 1= Yes 9= Unknown

19. Cancer 0= No 1= Yes 9= Unknown

20. Diabetes 0= No 1= Yes 9= Unknown

21. Chronic Obstructive Pulmonary Disease 0= No 1= Yes 9= Unknown

D. Clinical Characteristics

22. Presence of cavities as seen on CXR

0. No 1. Yes

23. Body Temperature

0. Above 38.5^0C 1. Below 38.5^0C

24. Cough

0. More than 2 weeks 1. Less than 2 weeks

25. Night sweat

0. No 1. Yes

26. Body weight loss

0. No 1. Yes