PREVALENCE OF TUBERULOSIS AMONG HIV-INFECTED CHILDREN AT MUHIMBILI NATIONAL HOSPITAL IN DAR ES SALAAM, TANZANIA.

By

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A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Medicine in Paediatrics and Child Health of the Muhimbili University of Health and Allied Sciences

November, 2009

CERTIFICATION

The undersigned certify that he has read and hereby recommend for the examination of a dissertation entitled *"Tuberculosis in HIV infected children below 14 years attending Muhimbili National Hospital in Dar es Salaam, Tanzania"* in fulfillment of the requirements for the degree of Master of Medicine (Paediatrics and Child Health) of the Muhimbili University of Health and Allied Sciences.

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Date.....18/11/09.....

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DEDICATION

I dedicate this work to all the children for better care,

To my family for all their prayers and support,

To My late father Cassian Njau,

To My dearest mother,

To my Sisters and Brother,

My nephew Alex and my niece Alice.

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ABBREVIATIONS

AFB	Acid-fast bacilli
AIDS	Acquired Immunodeficiency syndrome
ATS	American Thoracic Society
ARVs	Antiretroviral drugs
CDC	Centers for Disease Control and Prevention
CXR	Chest X-ray
CD	Clusters of differentiation
DNA	Deoxy nucleic acid
DOT	Directly observed therapy
DSM	Dar es Salaam
ELISA	Enzyme-linked immunosorbent assay
HIV	Human immunodeficiency virus
INH	Isoniazid
IPT	Isoniazid Preventive Therapy
LTBI	Latent tuberculosis infection
MIC	Minimal inhibitory concentration
MDR	Multi–drug-resistant
MTB	*Mycobacterium tuberculosis*
PCR	Polymerase chain reaction
PPD	Purified protein derivative
TB	Tuberculosis
TST	Tuberculin skin test
UNAIDS	Joint United Nations Programme on HIV/AIDS
WHO	World Health Organization

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PPD	Purified protein derivative
TB	Tuberculosis
TST	Tuberculin skin test
UNAIDS	Joint United Nations Programme on HIV/AIDS
WHO	World Health Organization

DEFINITION OF TERMS

Sputum confirmed tuberculosis	the presence of acid-fast bacilli on sputum microscopy and/or *Mycobacterium tuberculosis* cultured
Good clinical response	symptom resolution and weight gain of at least 5% of body weight at diagnosis within a month of starting antituberculous drugs.
TB contact/exposure	the child has had recent and substantial contact (living under the same roof for at least two weeks) with an adult or adolescent with contagious pulmonary TB (a source case).
Probable tuberculosis	presence of suggestive clinical manifestations and chest X-ray findings and/or good clinical response to antituberculous treatment in the absence of bacteriological confirmation.
Advanced HIV-associated immunodeficiency	is when age-related CD4 values are 25-30% in children aged 0-11 months, 20-25% in children aged 12-59 months and 200-349 in children above 5 years of age.

Severe HIV-associated immunodeficiency	is when age-related CD4 values are below 25% in children aged 0-11 months, below 20% in children aged 12-59 months and below 15% in children above 5 years of age.

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SUMMARY

Background: Tuberculosis (TB) has re-emerged as one of the major worldwide public health problem with increasing incidence in both adults and children, and that TB-infected children serve as a reservoir of the infection. It was estimated that there were over 700,000 people with HIV-TB co-infection in 2006 of whom about 200,000 died. WHO estimates that only half of all persons with smear-positive tuberculosis are identified. Diagnosis of TB in children usually follows discovery of an adult case, and relies on clinical manifestations, sputum examination and chest radiograph. However, clinical features are non-specific, chest radiographs are difficult to interpret, and routine laboratory tests are not helpful. These are worse in HIV-TB co-infection. Even though more rapid and sensitive laboratory testing have been discovered, results for children have not been successful.

Objectives: To determine the prevalence of tuberculosis among HIV-infected children aged between 2 months up to 14 years attending Muhimbili National Hospital in Dar es Salaam, Tanzania.

Materials and Methods: A cross-sectional study was conducted among HIV-infected children from 2 months up to 14 years of age at Muhimbili National Hospital, in Dar es Salaam, Tanzania, between July 2008 and January 2009. After obtaining informed consent, information on socio-demographic and anthropometric characteristics was collected using a structured questionnaire. History taking and physical examination was done for each child. Thereafter, tuberculin skin tests were performed and chest radiographs taken. Three consecutive sputum specimens were collected for examination, and blood sample was taken for CD4+ T lymphocyte percentage testing.

Results: Of the 182 HIV-infected children enrolled in the study, 104 (57.1%) were males. Thirty seven (20.3%) had TB diagnosed through clinical manifestations, chest

radiograph findings and/or sputum microscopy. Of the 37 children with TB, 33 were diagnosed by clinical manifestations and chest x-ray, 2 by clinical manifestations alone and 2 by clinical manifestations, chest x-ray and positive sputum results for AFB. About one percent had sputum smear positive TB and 19.2 % had sputum smear negative TB respectively. Positive sputum smear by microscopy was found in 5.4% of children with TB. The prevalence of TB was high in males (78.4%) compared to females. There was a higher proportion of TB (45.9%) in the age group below 24 months compared to other age groups. The commonest clinical manifestations were prolonged cough, fever, generalized non-tender lymphadenopathy and failure to respond to conventional antibiotic therapy with 51.4% children presenting with four or more symptoms and signs. One infant aged six months and one child with 37 months had cavitations. Thirteen (35.1%) of underfive children with TB had CD4+ T lymphocyte below 20%, and six (16.2%) children aged 5 years and above with TB had a CD4+ T lymphocyte below 15%. Out of 37 children with TB, 22(59.5%) were not on ARVs. Low CD4+ T lymphocyte percentage indicating advanced HIV-associated immunodeficiency and severe HIV-associated immunodeficiency were associated with increased risk of acquisition of TB in children. Children on ARVs were less likely to have TB as compared to those not on ARVs. Advanced HIV-associated immunodeficiency, severe HIV- associated immunodeficiency and positive TB contact were found to be significant predictors for TB.

Conclusions: One-fifth of children had HIV-TB co-infection. HIV-positive children with four or more clinical manifestations, low CD4+ T-lymphocyte percentage and advanced HIV disease were more likely to have TB.

Recommendations: Combination of clinical manifestations, radiological findings and sputum examination still be used to diagnose TB in HIV-infected children. History of open TB contact should be enquired routinely whenever HIV-infected children attend health care facilities. Follow up study should be done in children with low CD4+ T-lymphocyte percentage and advanced HIV disease to decide on their use as additional criteria for TB suspicion and aid TB diagnosis in HIV-infected children.

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1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 EPIDEMIOLOGY

In 2007, there were an estimated 9.27 million incident tuberculosis (TB) cases and 13.7 million prevalent TB cases worldwide. [1] Of the 9.27 incident cases of TB, an estimated 1.37 million were HIV-infected, of which 79% of these cases were in Africa.[1] In the same year an estimated half a million cases had multi-drug resistance (MDR) TB. Among the HIV-positive incident cases of TB, there were 456,000 deaths. [1] Furthermore, about one million (11%) of the annual TB global cases occur in children below 15 years of age. As in adults, drug-resistant TB is increasing. Still, many unanswered questions in the diagnosis and treatment of paediatric HIV-TB co-infection have remained.[2]

Thus TB remains one of the biggest ailments for people in the developing world. Diagnosis, treatment, and overall management of TB in adults have been documented well and tested in most countries.[1] For children, problems of diagnosis are much greater. This is because in TB programmes in most areas of the world children are not a priority, and hence making the diagnosis more difficult, resulting in a big number of cases being managed incorrectly.[1]

Most cases of human TB are caused by the human tubercle bacillus, *Mycobacterium tuberculosis*, but in countries where cattle tuberculosis still occurs human TB is also caused by *M bovis*.[3]

TB was considered a passing historical significance to clinicians more than a decade ago, but in 1985, due to primarily the newly recognized HIV pandemic, TB resurfaced.[4] Due to extensive transmission of TB around the world, the World Health Organization (WHO) declared TB a global emergency in 1993. [5]

In 1991, virulent MDR strains of *Mycobacterium tuberculosis* were reported by the Centers for Disease Control and Prevention (CDC).[6] The strains produced fatal and

fulminant disease among HIV patients, and also proved highly infectious in exposed health personnel.[6]

Despite effective control measures and chemotherapy, the prevalence of TB is still rising. The unprecedented growth of TB epidemic in Africa is attributable to several factors, the most important being HIV.[7] In Africa, TB is the commonest coexisting condition in people dying of AIDS.[7] A rise in numbers of infected children in the region would be expected, as these children are in close contact with their caregivers, who may be co-infected with HIV-TB.[8,9]

Human immunodeficiency virus (HIV) is a retrovirus that leads to *acquired immunodeficiency syndrome* (AIDS) in which the immune system fails, [10] leading to immunosuppression and development of life-threatening opportunistic infections.[10, 11]

Infection with HIV occurs by the transfer of infected or contaminated blood, semen, vaginal fluid, or breast milk. The HIV primarily infects vital cells in the human immune system such as helper T cells (specifically CD4+ T lymphocytes), macrophages and dendritic cells. HIV infection leads to reduced levels of CD4+ T lymphocytes by directly viral killing of infected cells, by increasing rates of apoptosis in infected cells, and by killing of infected CD4+ T lymphocytes by CD8 cytotoxic lymphocytes. When CD4+ T lymphocyte numbers falls below a critical level, cell-mediated immunity is lost, and the body eventually becomes more susceptible to opportunistic infections. [12, 13, 14] Untreated, eventually most HIV-infected individuals develop AIDS and die; however few remain healthy for years, with unnoticeable symptoms. Treatment with anti-retroviral drugs (ARVs) increases the life expectancy of people infected with HIV. [12, 13, 14]

HIV infection is now pandemic. The Joint United Nations Programme on HIV/AIDS (UNAIDS) and the WHO estimated that AIDS has killed more than 25 million people since first recognized in 1981. In 2006, it was estimated that 39.5 million adults and 2.3 million children under 15 years of age were living with HIV.[12]

The pandemic has a substantial impact on the clinical presentation, management and public health issues relevant to TB. [4] TB is unique among opportunistic infections in

individuals with HIV because it is transmissible to immunocompetent people via the respiratory route, can be easily treatable once diagnosed, may occur even in early-stages of HIV infection,[3, 4] and is preventable with drug therapy. However, multidrug resistance is a potentially serious problem, even though its incidence is being controlled by the use of directly observed therapy (DOT).[1, 4, 6]

The extent of the problem of HIV/AIDS/TB double infections in Sub-Saharan Africa has been described in the previous studies, of which Kenya, Ethiopia, South Africa and Tanzania ranked higher in the list of top 22 TB-endemic countries in WHO reports. [1, 10] While in some other parts of the world breakdown of control programs has contributed greatly to increased incidence, increases in Africa are mainly attributable to the HIV/AIDS epidemic. [1, 7, 15]

TB may develop at any point during the course of HIV infection, hence may occur early before significant drop in CD4+ T lymphocyte counts or other clinical conditions indicating HIV infection or AIDS. Presentation and radiological features depend on degree of immunosuppression.[4] In those with good immunity and CD4+ T lymphocyte counts, and low viral load, the presentation of TB is similar to those in HIV-negative individuals. In advanced HIV infection, TB often presents atypically as extra pulmonary or systemic disease. [4] Constitutional symptoms that are not localized to one particular site, often affecting bone marrow, bone, urinary and gastrointestinal tracts, liver, regional lymph nodes, and the central nervous system occur. Alternatively, symptoms may relate more to organ-system involved.[4, 16, 17]

The gold standard for diagnosis of TB is microscopy followed by culture. Molecular diagnostics may be valuable in reducing time spent in isolation facilities when TB is suspected clinically.[8, 18] Confirmation, by molecular diagnostics that acid-fast bacilli (AFB) are not *Mycobacterium tuberculosis* (MTB) may be useful for clinical management and infection control. [8,11-19]

Most recently developed sensitive and specific diagnostic tests are not routinely used for evaluation of children with suspected TB. Clinical criteria, particularly skin-test results, radiographic changes, and documented exposure to an infectious adult, remain standard diagnostic methods. [8,9,16,17] As new diagnostic tests are developed, they must be evaluated against clinical criteria before their application keeping in mind the differences in pathophysiology of TB in adults and children.[8]

There is considerable difficulty in establishing the diagnosis in children in general and in HIV-infected children in particular. This is because the common clinical criteria such as recurrent/chronic cough and failure to thrive may also be the result of other HIV related pulmonary infections such as disease due to environmental mycobacteria, especially by members of the *Mycobacterium avium* complex.[4, 16, 18] Likewise, the radiological characteristics of these other infections may be indistinguishable from those of TB. Thus, in the developing countries, misdiagnosis is common; rendering it difficult to assess magnitude of HIV related TB in children. A necropsy performed on HIV positive children in Bulawayo, Zimbabwe, established a TB diagnosis of five percent.[20]

1.2 PATHOPHYSIOLOGY OF TUBERCULOSIS

Humans are the only known reservoir for *Mycobacterium tuberculosis*. TB is transmitted by airborne droplet nuclei, [3, 21] which may contain fewer than 10 bacilli. The natural history of TB in children is a continuum, and it is better to consider three stages, which are exposure, infection and disease. [8] Exposure to TB occurs by sharing common airspace with a patient who is infectious. When inhaled, droplet nuclei are deposited on the alveoli. Upon encountering the bacilli, macrophages ingest and transport the bacteria to regional lymph nodes. TB mainly affects the lungs although other organs/systems can be affected.[2,3, 9, 21,22,23]

Deposited tubercle bacilli in the alveoli is associated with the following outcome: they may be killed by cells of the immune system, they may multiply and cause primary TB,

they may become dormant and remain asymptomatic, or the bacilli may proliferate after a latency period (reactivation disease).[21-23]

A single cough can generate 3000 infective droplets. Fewer than 10 mycobacterial bacilli may initiate a pulmonary infection. The primary focus can occur in any of the lobes of the lung, and a child can also have more than one primary focus. In most cases, the infection is contained at this stage and the children do not develop disease. In children in whom the disease is contained, hypersensitivity to tuberculoprotein develops which is demonstrated by the development of a positive tuberculin (Mantoux) skin test. However, in some children, the infection continues to proliferate. The hilar lymph glands enlarge and can be seen on chest radiography. Further progression leads to involvement of the adjacent bronchus. The radiological picture of childhood TB is the result of the primary focus and complications caused by the enlarged mediastinal lymph glands. The degree of involvement of the bronchus by the lymph glands leads to a wide variation in the radiological pictures. The primary focus can enlarge sufficiently eventually developing central necrosis, and this can lead to cavitation. Cavities although uncommon do occur in children.

TB inoculation can result in latent infection or active disease. Depending on the population, 10-30% of inoculated individuals progress directly to active primary disease. More commonly, however, TB inoculation results in an asymptomatic latent infection. Skin test conversion and identification of a Ghon's complex on chest radiography are means of identifying such cases.[8,16,17,21]

Some exposed children are infected with *M. tuberculosis, however* the clinician cannot know immediately which exposed children are infected because the development of delayed hypersensitivity to tuberculin may take up to three months.[8,17,24] Young children in the exposure stage should receive chemotherapy, usually isoniazid, after excluding active disease.[2, 8, 19]

TB infection is signalled by a reactive Mantoux TST.[8,14] In this stage, there are no signs or symptoms, and the results of the chest radiograph are either normal or show only

fibrotic lesions or calcifications in the lung parenchyma or regional lymph nodes. In developing countries, TB infection is rarely discovered and mostly is not treated.[8,17]

TB disease occurs when clinical manifestations or radiographic changes caused by *M. tuberculosis* appear.[8,19] Radiographic abnormalities and clinical manifestations in infected children probably are influenced more by host inflammatory reaction than by number of organisms present. [8,15,22] Studies show that in 40% to 50% of infants with untreated infection TB disease develops within 1 to 2 years, and the risk declines to 15% in older children. In a quarter of children TB is extra pulmonary and difficult to confirm.[8,15,22,24]

Patients often remain healthy for years; however, some experience reactivation of their disease due to subsequent immunologic stressors. [2, 21] Such reactivation occurs at a rate of approximately one percent per year in immunocompetent hosts. The conversion rate is 10% in immunocompromised individuals. Asymptomatic, patients with latent TB infection are not contagious. These qualify for isoniazid prophylaxis, which significantly reduces the risk of future TB reactivation.[21,22,24]

Roughly 80% of TB cases is pulmonary tuberculosis (PTB), though the disease can involve any organ system. In patients who are severely immunocompromised, extrapulmonary tuberculosis (EPTB) and atypical presentations are common.[9, 16,18,20,22] Treatment markedly reduces infectivity. The first dose of medication reduces the bacillary load by about 10-fold and therapy for 2 weeks reduces the bacillary load by a 100-fold factor.

1.3 TB-HIV Co-infection

The HIV pandemic has had a devastating effect on TB, especially in Sub-Saharan Africa.[1,2,5,12] Health care facilities in many African countries are overwhelmed by the additional burden of HIV related TB and urgent action is needed to stem this epidemic before it becomes uncontrollable.[1, 2, 8,13,14,18]

In short, the resurgence of TB over the last decade has coincided with the HIV pandemic. HIV-infected infants and children are in close contact with their caregivers, who may be infected with HIV and *M. tuberculosis* and are at high risk of developing infectious TB as they become immunocompromised.[8, 16] Of the available diagnostic tests for TB infection or disease in children, none has been evaluated systematically in children with HIV infection.[8,16,24,25]The sensitivity of diagnostic tests relying on host's immune response, such as the TST or serology are likely to be lower in HIV-infected children with TB.[8,21,24] Tests that directly detect *M. tuberculosis*, such as polymerase chain reaction (PCR) or antigen detection assays, may be particularly important for HIV-infected children due to diagnostic difficulties encountered. The culture yield of *M. tuberculosis* from children with HIV infection and TB is unknown but appears to be similar to that from non-HIV-infected children.[8, 19, 24] The most important diagnostic clue for detecting TB in HIV-infected children is a history of contact with an adult with an open TB.[8,11,16,26] However TB may not have yet been diagnosed in this adult, thus rapid and aggressive evaluation for TB in adult caregivers is a crucial part of evaluation of the child.[8, 10, 16-19, 22,

The current prevalence of drug resistance among *M. tuberculosis* isolates in the United States is 8% to 14%.[8, 27]Drug resistance is seen more in patients who received treatment, and are not responding to standard anti-TB drugs and or do not adhere to treatment. Children exposed to such adults have increased risk of drug resistant infection. Hence, drug-resistant TB is increasing among children. Due to low culture yields from children with TB, clinicians often rely on antimicrobial susceptibility results obtained from the adult source case that presumably infected the child. This reemphasizes the critical need to actively look for and evaluate the source case for every child suspected of having TB. Rapid identification of drug-resistant mycobacteria is necessary for prevention of drug-resistant TB. [6, 8, 18, 28]

Important determinants of a child's risk of developing TB after primary infection are age, with the highest risk occurring in children below three years of age, and the immune status of the child. The comparative risk of an HIV-infected child to develop TB is mainly influenced by the degree of immunosuppression and/or the severity of HIV disease.[4,10,14,24, 26]

Reported seroprevalence of HIV in children with TB ranges from 10 to 60%,[27] and in Tanzania 12-68% of children diagnosed with TB also have HIV infection. [28]

In one study, incident case rates of TB disease among HIV-exposed or -infected children were reported to be higher than those of comparable age in the general population.[14, 26] Also studies indicate an increased risk for TB disease among HIV-infected children in hospitalized African children. [29-32.]

Kawo et al did a study in year 2000 whose aim was to determine the prevalence of HIV-1 infection, the clinical spectrum of associated conditions and HIV-1-associated mortality among children hospitalized in the medical paediatric wards at Muhimbili Medical Centre (MMC), in Dar es Salaam, Tanzania. TB (19.3%) was among the three most common associated conditions. [29]

A two year prospective study in children with persistent lung disease attending a tertiary paediatric clinic in Durban showed that, children in poor communities, may have undetected TB for months despite routine investigations and epidemiologically being a prevalent condition needing consideration in chronic diseases.[30]

In one study, the reported incidence of TB per 100 child years among all children of 16.3 (14.1-18.8), and 6.3 (4.8-8.1) for those receiving HAART, representing a crude incidence rate ratio (IRR) 0.39 (95% CI: 0.3-0.5). Overall few cases were confirmed, however 34% of TB cases were confirmed in children receiving HAART, compared to 18% not receiving HAART.[31]

TB epidemic is sustained in conditions of poverty and overcrowding, where exposure to *Mycobacterium tuberculosis* and the vulnerability to progress to active disease are increased.[3,21,22,31] Children contribute little to the maintenance of the TB epidemic, but they are greatly affected by it. An important example is from a recent report from Cape Town which indicated that children less than 13 years of age contributed 13.7% of the total TB disease burden.[31] Diagnosis of TB in children has never been easy, and the advent of HIV related TB, with many unusual presenting features, has added significantly to the diagnostic difficulties.[32] It is therefore a good practice to try to confirm the

diagnosis of TB using available specimens (sputum, gastric aspirate, ascitic-, pleural- and or cerebral spinal-fluid, lymph node biopsy/fine needle) and radiological facilities. [33-36] A study done in ambulatory HIV-infected adult cohort in Tanzania, between September 2001 and March 2004 revealed that patients on TB treatment were more often males, had a low median CD4+ T lymphocyte count and a higher rate of both prior TB and a TST $\geq$ 5 mm induration.[37] Abnormal chest radiograph in the absence of symptoms was the basis for treatment in half of the cases.[37] In the study, 31% of tested subjects had a positive AFB smear or mycobacterial culture, and these subjects met a study definition of definite or probable TB. DNA probes were positive for *M. tuberculosis* complex on all 36 isolates tested.[37]

EPTB and miliary TB are more common among younger children who do not have HIV infection; likewise they are likely to progress rapidly from infection to active disease and might not be recognized as having TB disease because they might have negative skin tests and fewer symptoms of disease. Congenital TB is rare but has been reported among children born to HIV-infected women with active TB. The true incidence and whether the rate is higher among these children compared with children born to non-HIV-infected women with active TB are unknown. [24, 38]

In children, who most often have primary disease, the interval between infection and disease can be several months to several years, and radiographic abnormalities often are not accompanied by symptoms; moreover, these children are rarely infectious. The major reason for separating infection from disease in children is that the perception affects the approach to treatment. TB infection is generally treated with a single anti-TB drug (Isoniazid) whereas active disease is treated with two or more drugs. The rationale for the difference in therapy is that the likelihood of emergence of resistance to a drug increases as the bacillary population increases. This distinction is somewhat artificial in children since infection and primary disease are parts of a continuum.[8, 21, 30-36,38]

When evaluating new diagnostic tests the basic differences between the pathophysiology of TB in adults and children should be considered. Among children with recent TB infection, active multiplication of mycobacteria occurs with or without the presence of

radiographic abnormalities or clinical symptoms. Careful consideration and investigation is required to determine if and how the results of these new tests should influence the definitions and treatment of TB infection and disease in children. Children at high risk of developing TB disease once infected includes children recently infected with *M. tuberculosis* (within the past 2 years) , HIV-infected and infants.[8,17,19,23,33-36]

1.4 TB Diagnostic Methods

TB may develop at any point in the course of HIV infection. [4,18,21,39]Although diagnosis has never been easy, and the advent of HIV related TB, with many unusual presenting features, has added significantly to the diagnostic difficulties (clinical manifestations are non-specific, CXR is difficult to interpret, TST has low sensitivity), it is good practice to try to confirm the diagnosis of TB using available specimens and laboratory facilities. Heightened clinical acumen remains the mainstay of diagnosis. [2, 8, 16,19, 42]

1.4.1 Clinical Signs and Symptoms

Diagnosis of TB in HIV-infected children with its unusual clinical manifestations has added to diagnostic dilemma. TB may occur early in the course of HIV infection, often before any significant drop in CD4+ T lymphocyte counts occur or other clinical conditions suggestive of HIV infection or AIDS appear.[4] The nature and clinico-radiological features of TB may or may not depend on the degree of immunosuppression. [4, 11, 16, 28, 32, 33]

In children with good immunity and CD4+ T lymphocyte counts, and a low viral load, TB manifestations in HIV-infected individual are essentially similar to those in HIV negative persons. As the immunocompetence decreases, there is an increasing incidence of atypical TB and diagnostic difficulties that are posed by non-specific clinical manifestations which may be confused with those of other HIV related infections. [2, 4, 16, 39, 40, 41]These atypical forms of TB include rapid progression of active disease and a high proportion of EPTB and disseminated TB.[4, 30-33, 40, 41]

The commonest manifestations seen are prolonged/recurrent fever, failure to thrive, generalized lymphadenopathy, recurrent respiratory infection and chronic/recurrent diarrhea. [24,27,29,30,32,41,42]

Studies have also shown that as immunity declines, which is reflected by a fall in CD4+ T lymphocytes there's an increased risk of progressing, acquiring or reactivating latent infection and thus end up with TB disease. Also use of ARVs have positive beneficial impact in preventing such progression.[43, 44, 45]

Various clinical scoring systems have been proposed on the basis of available information and tests. Although helpful, many of these systems are not validated[37] due to low sensitivity and specificity. Nevertheless, an abnormal radiograph, and a history of exposure to an adult with TB remains the most effective method for diagnosing TB in children.[2, 8,24,25,33,38]

Two scenarios lead the clinician to suspect that a child has TB disease. Infants are more likely to be symptomatic than older children with pulmonary TB. Common symptoms are cough, fever, and failure to gain weight, and the clinical signs are surprisingly meager. Presentation of EPTB is referable to the involved organ.[19, 22, 25, 27, 33] The sensitivity and specificity of clinical manifestations are extremely low and can lead to misdiagnosis when radiographs and other tests are not available. The second scenario is when a child has had significant contact with an adult with suspected- or confirmed- open TB. Usually the TST is applied first and if is reactive, a subsequent chest x-ray (CXR) or physical examination leads to discovery of early disease.[16, 17, 23, 33, 34]

1.4.2 Tuberculin Skin Test (TST)

The Mantoux test (TST), which uses five tuberculin units of purified protein derivative, is the standard method for detecting infection by *M. tuberculosis*. Before the era of HIV infection, 75–85% patients with newly diagnosed pulmonary TB had a positive response to 5 units (intermediate strength) PPD testing. In HIV-TB co-infection there is a

reduction in the proportion of those reacting to PPD as the CD4 count falls, and therefore it limits the usefulness of the TST as a diagnostic tool.[8,16,19,23]

The reaction is measured as millimetres of induration after 48 to 72 hours.[8,23,24,34,38] TST is a way to determine asymptomatic infection by *M. tuberculosis*, thus a negative TST does not rule out TB disease in a child.[8] The rate of false-negative TST is higher in those who are tested soon after becoming infected with severe TB; in children with debilitating or immunosuppressive illnesses, malnutrition, or viral and certain bacterial infections; in infants; and if poor technique is used. The rate of false-negative TST in children with TB who are infected with HIV is unknown, but it is certainly higher than 10%.[8, 24, 34,38]

False-positive reactions to TST are often attributed to asymptomatic infection by environmental nontuberculous mycobacteria (NTM). In several studies, most children who received Bacille Calmette-Guérin (BCG) vaccination as infants have a nonreactive TST at 5 years of age.[8, 44] It was also observed that most older children or adolescents who receive BCG develop a reactive skin test initially; however, about 80% to 90% have lost tuberculin reactivity 10 years latter.[8,34,38]

1.5.3 Radiological Studies

Evidence of pulmonary TB in chest radiographs varies, but usually radiographs show enlargement of hilar, mediastinal, or subcarinal lymph nodes and lung parenchymal changes.[33, 36] Most of the radiographic abnormalities are caused by a combination of lung disease and the mechanical changes induced by partial or complete airway obstruction resulting from enlarging intrathoracic nodes. The most common findings are segmental hyperinflation then atelectasis, alveolar consolidation, interstitial densities, pleural effusion, and, rarely, a focal mass. Cavitation is rare in young children but is more common in adolescents, who may develop reactivation disease similar to that seen in adults.[19,21,35,36]

As of today, there is no routine role of computerized tomography (CT) scan in evaluating an asymptomatic TB-infected child with a normal chest radiograph. This scan can be

helpful in selected cases to demonstrate endobronchial disease, pericardial invasion and bronchiectasis when the chest radiograph is abnormal but the pathologic process is not clear.[8]

The radiological findings in TB are neither specific nor diagnostic of TB. [8,33,36,38] Hilar adenopathy on chest radiography is the commonest disease manifestation in children with TB, and it is mostly regarded as the hallmark of recent primary TB. Lateral views and CT have been tried to improve the accuracy of diagnosis, but lateral CXR views have not been shown to improve the results and CT scan is useful in selected cases such as endobronchial disease and pericardial invasion when the pathology is unclear.[8, 33, 36]

1.5.4 Mycobacterial Detection and Isolation

Confirming the diagnosis of TB using available specimens and laboratory facilities is important. Direct microscopy and, if possible, culture of any specimens is important.[8, 16, 21, 38,40] Bacteriological confirmation is difficult in children, as there are fewer bacilli and obtaining clinical samples can often be difficult. Sputum is smear-positive in ≤10–15% of those with probable TB. Culture is the only way to differentiate *M.tuberculosis* from other mycobacteria and is particularly important if drug-resistance is suspected. [8, 16, 33]

Unfortunately, culture is positive in <40% of children with probable TB. Culture using solid media takes 4–6 weeks. [8, 14, 16, 38] Treatment is frequently initiated based on a presumptive TB diagnosis without waiting for the culture result. Rapid liquid-based cultures e.g. BACTEC 460 TB system (Becton Dickinson, Sparks, MD, USA) are available and can give results in 1–3 weeks. However, these are expensive and require specialist equipment and training, making them unavailable in many centres, particularly in the developing world. Appropriate samples for bacteriology include sputum, gastric aspirates and biopsy nodes or fine-needle aspirates.[8, 14, 16, 38]

Older children or adolescents suspected of having PTB may be able to produce sputum spontaneously. This is more likely to be positive than other samples. Three specimens are

recommended: one on the spot, an early morning specimen and a second on the spot at follow-up, and the last specimen on the spot which is then submitted to the laboratory.[38]

1.5.5 Gastric washings

Gastric aspiration using a nasogastric feeding tube (NGT) can be performed on young children who are unable to produce sputum. Morning samples are collected on three consecutive days. This usually requires admission to hospital to obtain samples following an overnight fast of about eight to ten hours. Even under optimal circumstances less than half of the samples will be positive in those with TB.[8, 16, 38]
The disadvantage is that gastric aspirates are uncomfortable, involve fasting and require repeated attendance or admission to hospital. Some studies have shown comparable results in the community, with suitable support, avoiding the need for admission. Nasopharyngeal aspirates which are less expensive can be used as an alternative. One study comparing the two techniques showed a yield of 30% in nasopharyngeal aspirate compared with 38% in gastric aspirates in the same unit.

The best specimen for culture from children with suspected PTB is the early morning gastric aspirate, the yield *M. tuberculosis* is usually in only 30% to 50% of cases. The culture yield from other body fluids or tissues from children with EPTB is usually less than 50%.[8, 34, 38]

1.4.6 Other methods

Although newer methods, such as PCR-based detection systems and DNA fingerprinting, are available and are being refined, they have not yet been extensively validated in children. The expense and the facilities required are likely to preclude their use in areas where TB is endemic, and the need for new diagnostic tests for TB has been repeatedly emphasized.[8]

Despite advances in technology, early bacteriologic diagnosis of TB still relies primarily on examination of acid-fast-stained smears from clinical specimens. However, young

children with PTB rarely produce sputum and are usually not able to expectorate voluntarily. When sputum samples cannot be obtained, gastric aspirate samples are used for detection and isolation of *M. tuberculosis*. The rates of positive AFB stain from body fluids and tissues in children with EPTB also are low, and false-positive results caused by NTM disease are common.[8, 24, 25, 34, 38]

1.4.7 New Diagnostic Techniques

Diagnostic PCR is a technique of DNA amplification that uses specific DNA sequences as markers for microorganisms. Different PCR techniques, most using the mycobacterial insertion element IS6110 as the DNA marker for *M. tuberculosis*-complex organisms, have a sensitivity and specificity greater than 90% for detecting PTB in adults. [8, 34] Although PCR yield is higher than that of smear, and provides a rapid test in a child suspected to have TB, its major role will be in evaluating children with significant pulmonary disease, when the diagnosis is not easily established on clinico-epidemiologic grounds. Furthermore, performing PCR on gastric aspirates is not a useful test to distinguish between TB infection and disease and thus should not be used for children with normal chest radiographs.[8, 41]

Serology has found little place in the routine diagnosis of TB in adults or children. Several recent studies have used the enzyme-linked immunosorbent assay (ELISA) to detect antibodies to various purified or complex antigens of *M. tuberculosis* in children.[8, 23] So far, no available serodiagnostic test for TB has adequate sensitivity, specificity, or reproducibility under different clinical conditions to be useful for diagnosing TB in children. Measurement of tuberculostearic acid, has been employed to detect *M. tuberculosis* in clinical specimens; however, these techniques require advanced equipment and their sensitivity and specificity in children are not known. [8]

Using mycobacteria-specific, radioisotope-labelled systems (Radiometric BACTEC 460 TB and BACTEC MGIT 960 System for clinical specimen other than blood) help establish the diagnosis of active TB and are recommended in patients infected with HIV.

Such tests should be used for all patients with HIV who are suspected of having TB, as bacillemia is particularly prevalent in this population.[8,19,21,34,38]

1.5 Diagnosis

Because of the difficulty of definitively diagnosing TB disease among children, a high index of suspicion is important. The diagnostic problems are more pronounced in HIV-infected children and current diagnostic algorithms work poorly in this subgroup of children due to different factors.[7-11, 14-16, 25, 29, 38-43] A definitive diagnosis of TB disease requires isolation of *M. tuberculosis* from expectorated sputum, bronchoalveolar lavage fluid, early morning aspirated gastric fluid, biopsied lung, peripheral lymph node or other tissue, or mycobacterial blood culture. [8, 19,21] Antimycobacterial drug susceptibility testing should be performed on the initial *M. tuberculosis* isolate and on subsequent isolates if treatment failure or relapse is suspected.[6, 18, 21, 40, 43]

The Tanzanian National TB and Leprosy guideline for diagnosis of TB in children recommends clinical findings, family history of contact with a smear positive case, X-ray examination and tuberculin testing, culture (if available) and non-response to broad spectrum antibiotic treatment. For older children who are able to cough up sputum, they should be assessed using smear microscopy as the gold standard (appendix).[38]

1.6 Statement of the Problem

HIV-infected children are in close contact with their caregivers, who may be infected with both HIV and TB, and are at high risk of developing active TB as immunity decreases. In non-HIV infected children the sensitivity of diagnostic tests relying on host's immune response like TST may be suppressed if they have malnutrition or other immunosuppressive conditions, it is likely that the tests' sensitivity also will be lower in HIV-infected children with TB. The culture yield of *M. tuberculosis* from children with HIV infection and TB is unknown but appears to be similar to that from HIV-uninfected children. Furthermore, this gold standard method of confirmation of *M. tuberculosis* infection on culture is often not commonly attempted or achieved. This is because of difficulties in obtaining sputum and the conventional culture method takes a long time (six weeks) for results which will lead to delay in treating a sick child if results are to be waited. Also, there is an increase in drug resistance in adults, and children exposed to adults are at increased risk of having drug resistant *Mycobacterium tuberculosis*. Chest radiograph is one of the tests used in the diagnosis of TB, but it has its own inherent problems, as there is a large intra- and inter-observer variability in interpretation of the radiograph.

The most important diagnostic clue for detecting TB in HIV-infected children is a history of contact with an infectious TB source case who usually is an adult. However TB may not have yet been diagnosed in such individual, thus aggressive evaluation for TB in adult caregivers is a crucial part when evaluating a child. Diagnosis of TB in children therefore relies on thorough assessment and appropriate investigations. Studies indicate that TB diagnosis requires time and a number of investigations (TST, CXR, sputum, gastric aspirate), because of the association with non-specific lung conditions that are common especially in HIV infected children.

Prompt and accurate diagnosis of TB is crucial because children especially the young ones living in Dar es Salaam are in an area with high prevalence of TB. In 2004, Dar es Salaam contributed to about 24% of all cases of TB in Tanzania, with a notification rate of > 200 (smear positive) per 2 million people. On the other hand, rapid culture methods

such as *M tuberculosis* blood culture and DNA PCR are available but are costly since they require the initial investments for the equipments, specialist training of laboratory personnel, and supplies. Thus, this study will shed light into prevalence of TB in HIV infected children.

2.0 OBJECTIVES

2.1 Broad

To determine the prevalence of tuberculosis among HIV-infected children aged 2 months up to 14 years at Muhimbili National Hospital in Dar es Salaam, Tanzania.

2.2 Specific

2.2.1 To determine the prevalence of TB in HIV-infected children by age and sex.

2.2.2 To determine the prevalence of TB by clinical manifestations, radiological findings and microbiological results of children with HIV.

2.2.3 To show the level of immunosuppression in relation to TB in the study population

2.2.4 To determine risk factors for TB in HIV-infected children in the study population.

3.0 Hypothesis

3.1 There is low prevalence of TB in HIV-infected children.

3.2 There is a relationship between the level of immunosuppression and TB in HIV-infected children.

4.0 MATERIALS AND METHODS

4.1 Study Design

This was a tertiary hospital-based descriptive study that was conducted between July 2008 and January 2009.

4.2 Study Population

The targeted population was HIV-infected children below 14 years of age. According to Population Reference bureau & UNAIDS, in Tanzania there were 140,000 children with HIV/AIDS by 2007 among 1.4 million people living with HIV/AIDS (PLWHA). At MNH, by end of December 2008 around 700 children had been registered of which about 485 were on ARVs. The study population was HIV-infected children aged 2 months up to 14 years who met the inclusion criteria.

4.3 Study Settings

The study was conducted at the Paediatric HIV and AIDS care and treatment clinic (CTC), outpatient department and in paediatric wards at MNH, in Dar es Salaam, Tanzania. Dar es Salaam is the commercial city of Tanzania located along the East coast of Indian Ocean and has Ilala, Temeke and Kinondoni municipalities. MNH is one of the biggest hospitals in the country and serves both as a referral and teaching hospital for the Muhimbili University of Health and Allied Sciences (MUHAS). The population of Dar es Salaam is estimated to be 3.8 million (2007), of which 44% were children aged 0-14 years (Tanzania Bureau of Statistics).

4.4 Inclusion Criteria

The inclusion criteria were HIV-infected children aged 2 months up to 14 years with cough and or fever for at least two weeks and or weight loss; confirmed either by HIV ELISA for children 18 months and above or HIV DNA PCR for children below 18 months of age, and child whose parents/guardians consented to participate in the study.

4.5 Exclusion Criteria

The exclusion criteria included parent/guardian who did not consent his/her child to participate in the study, children below 2 months and those aged 14 years or above, and a child already on anti-TB therapy so as not to interfere with laboratory results or with previous history of TB treatment.

4.6 Sample Size

The sample size of the study population was calculated using the following formula:-

$n= Z^2 P (100- P)/ E^2$

Where n= calculated sample size

Z= critical value of 1.96 corresponding to 5% significant level.

P=prevalence (taken as 19.3% based on the 2000 study conducted at MNH, formerly MMC).[29]

E= Margin of error 6%

$n= (1.96)^2 \times 19.3\% (100-19.3\%) / (6\%)^2$

The calculated sample size was 166.2 children, thus the minimum sample size was 166 HIV infected children.

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4.7 Sampling Procedure and Recruitment of Study Participants

Convenient sampling procedure was used. During the study period all children with known HIV positive serostatus and meeting the inclusion criteria admitted to the paediatrics wards were included in the study after consent was obtained from their parents, in which recruitment was done throughout the week. For children attending CTC clinic, the first five children who parents consented attending the clinic every Wednesday were recruited consecutively after meeting the inclusion criteria. This was the allocated day for conducting the study so as not to interfere with routine ward work and attendance to other clinics and for completion sample size in time, and the average number of recruited children was due to logistics of doing CXR and sputum. Overall, five cases was recruited per day and sample collection done, until a sample size of 182 children was reached.

4.8 Data Collection and Instruments Used

A structured questionnaire designed for the purpose of the study was used. The questionnaire was structured to obtain information on socio-demographic, anthropometric, clinical presentation in which parents/guardians were asked about the history of TB contact, fever and cough, previous use of antibiotics, and weight loss or growth faltering. History of the child being on cotrimoxazole prophylaxis or antiretroviral therapy (ART) was also obtained.

Clinical examination was done according to Hutchison's Principles of Paediatric and recorded. This included taking the patient's weight and height and the child's present HIV disease stage. Basis of the TB diagnosis was recorded in the individual case report forms. Questionnaire was piloted prior to actual data collection so as to test the practicability of the questionnaire.

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4.9 Diagnosis of TB

4.9.1 Clinical presentation

Complete history taking of prolonged/recurrent cough, fever, failure to thrive, TB contact and failure to respond to conventional antibiotic therapy was taken. Then, thorough, physical examination which included anthropometric measurements, general and then systemic examinations were performed. At one month additional data on clinical improvement for those initiated on anti-TB therapy was added. Time to event was not done. Conventional antibiotics was defined as are standard antibiotics used at appropriate dose and duration covering both 1stand 2nd line (which includes penicillins, co-trimoxazole, chloramphenical) and also according to severity, response to treatment and culture/sensitivity pattern if it was done.

Weight was measured by using a table SECA (seca gmbh & co. kg. Germany) scale for children below one year in the ward with a patient putting on light clothing and removing shoes. At CTC clinic and for children one year and above, they stood on solar cell SECA weighing scale. Weight was measured to the nearest 100 gm. Calibration of the scale was done before measurement. For accuracy and consistent data the scales were be checked weekly with standard weights. For height/lengths measurements, parents/guardians assisted in removing the child's shoes and gently laying the child in supine position on the length board with their heads placed at 90° with the head piece while the investigator straightened the legs, to ensure that the feet were at the right angles to the legs and brought the sliding foot piece into contact with the child's heels. For children $\geq$ 2 years who were able to stand, height was taken by standing upright on the board and the head piece was placed on the head at 90°. Height/length was recorded in the nearest 10 millimetres using local standardized height/length boards. Using WHO criteria Weight/Height Z-score (WHZ) was calculated. Z-scores were interpreted as follows: Z < -1.0 = mild malnutrition; Z< -2.0 = moderate malnutrition and Z< -3.0 = severe malnutrition.

4.9.2 Radiological investigation

Chest radiographs were done to all children. Anterior posterior view (AP) in supine/erect position or postero-anterior (PA) was taken. The CXRs were read and radiological findings reported by an experienced specialist radiologist.

4.9.3 Microbiological investigation

The TST (Mantoux test) was done to 161 children by injecting 5 tuberculin units (TU) of purified protein derivative (PPD) intradermally in the anterior aspect of the forearm. During the injection of a TST, universal precautions were adhered, that is the anterior surface of the forearm below the elbow was cleaned with an alcohol and allowed to dry. A disposable syringe and needle was used to draw the reagent. Thereafter, 0.1 mL (5 TU of PPD) was administered. The parents were instructed not to rub or scratch the site of the TST test and to keep area clean. Children were asked to return in 48 to 72 hours for determination of the size of the induration. The decision that the reaction was significant was based on size of induration in millimetres (from 5 mm onwards since this is a cohort of HIV-positive children). None came in 48 hours. If a child was brought back after 72 hours following TST administration and the induration was > 5 mm, it was considered as a positive TST. Children returning after 72 hours with no measurable induration, results were considered as invalid.
Three consecutive sputum or early morning gastric aspirate by inserting nasogastric tube (NGT) specimens were obtained for microscopic examination of acid-fast bacilli (AFB) and for culture of tubercle bacilli. Children aged five years and above and able to give out sputum were instructed to cough out the sputum. Seventy two children of whom 13 were too sick to feed and 59 below three years of age, collection was done by NGT. After detailed explanation of the insertion procedure, together with the reasons why the tube is used verbal consent was obtained. The tube was measured from the tip of the nose to the ear and then down to the breastbone, and was then dipped in water. Thereafter the NGT was inserted through the nostril until the marked point was reached, and when it was properly placed it was taped to the child's nose and face without blocking the nostril. Sputum induction was not done. For those between three and five years of age and able to cough out sputum and

follow instructions they were instructed on how to give out the sputum, The sputum specimen was collected from 158 children in sterilized plastic container, decontaminated by 4% NaOH and concentrated by a Cruickshank technique. Smears were prepared, stained by Ziehl-Neelsen (ZN) technique and examined using microscope for presence or absence of AFB. Children with ≥1 positive AFB smear were considered smear positive, and those with all negative AFB smears were considered smear negative. Sputum specimens were also inoculated into slopes of Lowenstein Jensen (LJ) medium and incubated at 35°C to 37°C. The LJ slopes were then examined for the growth of *M. tuberculosis* for eight consecutive weeks. Because of a low detection rate by these techniques, a positive result by microscopy or culture confirmed TB, but a negative result did not exclude the TB. In addition, three mL of venous blood was drawn from the anterior cubital fossa of each child using a sterile, disposable syringe and a needle. The child's skin was cleaned with 2% tincture of iodine and a 70% alcohol swab, and allowed to dry before drawing blood for CD4+ T lymphocyte percent testing using FACSCalibur system (Becton Dickinson, San Jose, CA). Laboratory testing for AFB microscopy, culture and CD4+ T lymphocyte percentage testing was performed at Central Pathology Laboratory, MNH. The CD4+ T lymphocyte was interpreted according to the national guideline (appendix).

4.10 Ethical Clearance

The study protocol was submitted to Institution Review Board and the ethical clearance to conduct the study was obtained from the Senate Research and Publication Committee of MUHAS. Permission to conduct the study was obtained from the MNH administration. During the course of the study, the study aims and procedures of the study were explained to the parents/guardians of the children. Written informed consent was obtained from parents/guardians prior to the enrolment of the child into the study. Study participants were identified by participant identification numbers to ensure confidentiality. Laboratory results were made available to the attending clinician managing the child, who would explain to the parents/guardians and recorded them in the individual case files. Children with life threatening infection were referred to paediatric wards within the Department of Paediatrics

and Child Health, MNH for further management and care. Children who had opportunistic infections received appropriate treatment and care. Children whose parents/ guardians did not provide consents for them to participate in the study received the similar quality of management and care as study participants since participation in the study was voluntary.

Risk benefits

Two children with positive TB contact but found not to have TB were referred to TB clinic for isoniazid (INH) preventive treatment (IPT). Current Tanzanian NTLP guideline for management of children in close contact with an infectious adult source case recommend IPT for children less than 5 years of age and HIV positive household contacts of TB patients after exclusion of active. The study thus provided an opportunity for an intensified TB control and prevention in children. Children with severe malnutrition (19) were referred to the ward for children with severe malnutrition for further management including admission for some while others received RUTF (plumpy nuts) to use at home, and to come for follow-up.

5.0 Data Analysis

Data were entered and analyzed using SPSS version 15.0 (Chicago, IL) and Epi Info version 6.4 (CDC, Atlanta). Distribution of baseline socio-demographic, anthropometric, clinical presentation characteristics, radiological, microbiological and immunological findings, and prevalence of TB were summarized in tables. Descriptive statistics were performed to depict overall distribution of the variables. Continuous variables were summarized using means, medians and standard deviations (SD). Chi-square test was used to compare differences in proportions. Finally, multiple logistic regression models were run to control for potential confounders. Odds ratio (OR) and their 95% confidence interval (CI) are presented as the risk estimator. p-value < 0.05 was considered significant.

6.0 RESULTS

During the study period, 182 children were enrolled, of whom 104 (57.1%) were males. Male to female ratio was 1.3. Of the total study participants, 87(47.8%) were underfives. The median age was 64 months (range 2 to 167 months). Overall, 19.2% of the children were orphans (mother not alive). Eighty five (46.7%) of children were from Kinondoni municipal, 54 (29.7%) from Temeke municipal, 41(22.5%) from Ilala municipal and two (1.1%) were from outside Dar es Salaam region. The median CD4+ T lymphocyte was 22%, and the mean was 22.79 (std. deviation 10.569).

Table 1: Baseline characteristics of the study population (N=182)

Characteristics	n (%)
Sex	
Male	104 (57.1)
Female	78 (42.9)
Age (months)	
2-23	37 (20.3)
24-59	50 (27.5)
60-119	59 (32.4)
120+	36 (19.8)
Mother (alive)	147 (80.8)
BCG immunization (Yes)	176 (96.7)
BCG scar present (Yes)	148 (81.3)
Severe malnutrition	19 (10.4)
Mild-moderate malnutrition	47 (25.8)
Normal nutritional status	116 (63.7)
Cotrimoxazole use (Yes)	172 (94.5)
ARV use (Yes)	122 (67.0)
% CD4+ T lymphocytes < 15%	43 (23.6)
% CD4+ T lymphocytes ≥ 25%	82 (45.1)
HIV (present) stage	
1	6 (3.3)
2	91 (50.0)
3	49 (26.9)
4	36 (19.8)

Of the 182 children recruited, 37(20.3%) were diagnosed to have TB using clinical manifestations, chest X-ray finding and/ or sputum examination. Two (5.4%) were diagnosed by clinical manifestations, chest x-ray and sputum results, 33 (89.2%) by clinical manifestations and chest x-ray, and two (5.4%) through clinical features only.

Overall, two children (1.1%) had bacteriologically confirmed TB and 28 (19.2%) had probable TB. At one month following initiation of antituberculous drugs, three children did not turn up and two did not show good clinical response to anti-TB therapy respectively. Also, one child developed jaundice and hepatomegaly and the anti-TB were stopped, and one child with positive sputum for AFB died before treatment.

Table 2: Prevalence of TB in the study population by age and sex

		Children with TB (n=37)	Children with no TB (n=145)	p-value
Variable	**Category**	**n (%)**	**n (%)**	
Sex	Male	29 (78.4)	75 (51.7)	0.003
	Female	8 (21.6)	70 (48.3)	
Age (months)				
	2-23	17 (45.9)	20 (13.8)	0.001
	24-59	7 (18.9)	43 (29.7)	
	60-119	8 (21.6)	51 (35.2)	
	120 +	5 (13.5)	31 (21.4)	

Twenty nine (78.4%) children with TB were males, with prevalence of TB being highest in the age group below 24 months (45.9%).

Table 3 shows that, among 37 children with TB, 97.3% had received BCG immunization and 73.0% had BCG scar. Cough of more than two weeks, fever of two weeks or more, failure to respond to conventional antibiotics, generalized lymphadenopathy, HIV WHO stage 3 & 4, low CD4+ T lymphocyte percent and not being on ARVs were found in more than 50% of children diagnosed to have TB. It was also found that in children diagnosed to have TB, 51.35% had four or more clinical manifestations.

Table 3: Clinical, radiological and microbiological findings of the study population

Variable	Category	TB +ve n=37 (%)	TB –ve n=145 (%)	p-value
BCG immunized		36 (97.3)	140 (96.6)	0.821
BCG scar present		27 (73.0)	121 (83.4)	0.145
Malnutrition	No malnutrition	19 (51.4)	97 (66.9)	<0.001
	Mild-moderate	6 (16.2)	41 (28.3)	
	Severe	12 (32.4)	7 (4.8)	
ARV use		15 (40.5)	107 (73.8)	<0.001
Cotrimoxazole use		34 (91.9)	138 (95.2)	0.434
+ve TB contact		7 (18.9)	2 (1.4)	<0.001
Cough	> 2 weeks	37 (100.0)	62 (42.3)	‡<0.001
	≤ 2 weeks	0 (0)	83 (57.2)	
Fever	≥ 2 weeks	20 (54.1)	16 (11.0)	<0.001
	< 2 weeks	17 (46.0)	129 (89.0)	
Weight loss	≥ 10%	10 (27.0)	4 (2.8)	‡<0.415
	No loss or < 10%	27 (73.0)	141 (97.2)	
No antibiotic response		23 (62.2)	13 (35.1)	<0.001
Lymphadenopathy		23 (62.2)	22 (15.2)	<0.001
+ve TST		5 (13.5)	1 (0.7)	‡0.001
HIV stage	1 & 2	5 (13.5)	92 (63.4)	<0.001
	3 & 4	32 (86.5)	53 (36.6)	
CD4+ T lymphocytes	< 15 %	11 (29.7)	32 (22.1)	<0.001
	15-24 %	19 (51.4)	38 (26.2)	
	25% and above	7 (18.9)	75 (51.7)	
Chest X-ray	Normal	2 (5.4)	111 (76.5)	<0.001
	Cavities	2 (5.4)	0 (0.0)	
	Lymphadenopathy	9 (24.3)	0 (0.0)	
	Features of pneumonia	15 (40.5)	11 (7.6)	
	Other findings	9 (24.3)	23 (15.9)	
+ve sputum smear		2 (5.4)	0 (0.0)	‡0.045

‡ p-value from fisher's T test; other p-value from χ^2 test for difference in proportions

The commonest manifestations encountered in those with TB were prolonged cough of more than two weeks in 37(100%), fever of two weeks or more 20 (54.1%), generalized non-tender lymphadenopathy 23 (62.2%) and failure to respond to antibiotic therapy 23 (62.2%). Mantoux test was performed in 161 children enrolled in the study. TST of five millimetres or more was encountered in five children among those diagnosed to have TB and in one child without TB (p=0.001).

Among children with TB, 22 (59.5%) children were not on ARVs, 32 (86.5%) were in HIV stage 3 & 4 and 11 (29.7%) had a CD4+ T lymphocytes below 15%.
There were significantly more children with advanced HIV disease and advanced-severe immunodeficiency among those diagnosed with TB compared to those without TB ($p < 0.005$). Sixteen out of 43 children with CD4+ T lymphocytes below 15% were not on ARVs. Advanced HIV- and severe HIV-associated immunodeficiency is when CD4+ T lymphocytes is below age related values as explained in definition of terms and in the methodology section. One child with TST of 5 mm or more induration had CD4+ T lymphocyte below 15%.

Table 3 also summarises CXR findings encountered in the study population. The abnormalities seen included features suggestive of pneumonia, infiltrates and hilar and or mediastinal lymphadenopathy and other findings (such as lung lobe collapse, miliary TB). Cavitations were seen in two underfive children, and miliary picture was seen in four children.

Positive sputum smear was found in 5.4% of those with TB (p=0.007).
At one month following initiation of anti-TB, 28 (75.7%) children had good clinical response.

Table 4: The level of immunosuppression in relation to TB

TB	Age groups (months)	% CD4+ T lymphocytes			
		Below 15	15-19	20-24	25+
Yes					
	0-11	0	2	2	2
	12-59	5	7	1	5
	60 and above	6	5	2	0
No	0-11	0	1	0	3
	12-59	11	5	10	33
	60 and above	21	8	14	39

Fourteen (37.8%) of underfive children with TB had CD4+ T lymphocytes below 20%, and six (16.2%) of children aged five years and above with TB had a CD4+ T lymphocytes below 15%.This is showing severe HIV-associated immunodeficiency.

Table 5: Risk factors associated with TB in the study population

Variable	n	TB positive (%)	TB negative (%)	OR (95% CI)	‡Adjusted OR (95% CI)
Male sex	104	78.38	51.72	3.38 (1.36-8.66)	3.58 (1.24-10.30)
BCG scar present	148	18.24	81.76	0.45 (0.21-1.36)	0.52 (0.16-1.64)
Severe malnutrition	19	63.16	36.84	9.46 (3.03-29.92)	1.43 (0.41-4.95)
Advanced-severe immunodeficiency	70	34.29	65.71	3.97 (1.75-9.13)	7.28 (2.34-22.64)
Advanced HIV infection	85	37.65	62.35	11.11 (3.82-34.72)	2.79 (0.89-8.73)
+ve TB contact	9	77.78	22.22	16.92 (2.99-124)	8.11 (1.26-52.19)
ARV use	122	12.30	87.70	0.24 (0.11-0.55)	0.41 (0.16-1.07)

Table 5 summarises risk factors associated with development of TB. Among which severe malnutrition, advanced HIV and severe HIV-associated immunodeficiency [OR; 3.97 (95% CI: 1.75-9.13)] and advanced HIV (stage 3 or 4) infection [OR; 11.11 (95% CI: 3.82-34.72)] showed increased likelihood of association, while ARV use [OR; 0.24 (95% CI: 0.11-0.55)] showed protective effect. When multiple regression model was run, only positive TB contact [Adj OR 8.11 (95% CI: 1.26-52.19)] and advanced HIV- and severe HIV-associated immunodeficiency [Adj OR 7.28 (95% CI: 2.34-22.64)] showed increased risk for TB respectively.

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7. DISCUSSION

The study aims were to determine the prevalence and to describe the clinical manifestations, radiological findings and microbiological results of TB among HIV-infected children between two months up to 14 years of age attending Muhimbili National Hospital in Dar es Salaam, Tanzania.

Findings from this study showed that the prevalence of TB was 20.3% by using clinical manifestations, radiological findings and/or microbiological results. The results concur with a study done by Kawo et al between August 1995 and January 1996 in this same setting in which TB was diagnosed in 19.3% in HIV-infected children.[29] It is also in close consistency with that from a post-mortem study that was done in Zambian children in which *M.tuberculosis* was found in 18% of children with HIV.[47] This could be a reflection of a true prevalence of TB in HIV infected children. Nevertheless, the Zambian study was a necropsy hence presentation, radiological findings and sputum examination not studied. For this reason, the prevalence was obtained but diagnostic criteria for children with TB is not known thus cannot be teased out to aid clinicians in reaching diagnosis in HIV-infected children. However, the prevalence from this study is higher than that observed by Rennert et al in Soweto between October 1998 and April 1999, in which a TB prevalence of 4% was found in children below 6 years.[32] The difference could be attributed to probability of successful TB treatment before death of a child, because from literature the prevalence of TB is expected to be high in young children.[4,10,11,14,21,24,26] Among 37 children with TB, two were diagnosed using clinical criteria only and showed at one month following initiation of anti-tuberculous drugs.

There was a statistically significant difference in the prevalence of TB between male and female children in this study. Out of 37 children with TB, 78.4% were males, and TB prevalence was 45.9% in the age group below 24 months. These findings are in agreement with previous findings from a study done in ambulatory adult population in Dar es Salaam, Tanzania, between September 2001 and March2004, which revealed that TB patients on treatment were more often males with a low median CD4+ T lymphocyte count. [35] However there are no conclusive remarks from studies indicating that male sex is a risk factor for TB.

TB prevalence in our study was highest in the age group below 24 months and decreased thereafter. Previous studies documented that the most important determinants of a child's risk of developing TB after primary infection are age with the highest risk occurring in children below three years of age and the immune status of the child.[11, 21-24,26,43] Among the reasons is the fact that the immaturity of both innate and adaptive immune systems of young children plays an important role in augmenting susceptibility to *M. tuberculosis* disease. [22] Using clinical manifestations, 51.4% children presented with four or more clinical manifestations. There was significant differences in radiological findings between children with TB and those with no TB ($p<0.001$). History of positive TB contact, advanced HIV- and severe HIV- associated immunodeficiency and advanced HIV disease were found to be significant risk factors for TB while use of ARVs was found to be associated with decreased risk for TB. Twenty five (68%) children with TB had been diagnosed with HIV within the past 18 months.

Cough of more than two weeks, fever of two weeks or more, failure to respond to conventional antibiotics, generalized non-tender lymphadenopathy, HIV stage 3 and 4, low CD4+ T lymphocytes percent and not being on ARV were the commonly encountered in children with TB. This is in consistency with a study by Temiye et al in which majority of the subjects had four or more symptoms and signs. [10].

History of positive TB contact was found to be a significant risk factor for TB in HIV-infected children. This agrees with different studies done in different places.[1,8,21,25,38] Ikeogu et al, Marais et al, and Lolekha et al found some of these manifestations, and thus the manifestations commonly found in our study can be utilized to help in diagnosis of TB in HIV infected children, or where HIV is endemic.[11,20,24,43]

Within the households of the TB cases, the TST positivity was not higher compared to non-contacts. The risk of TB infection in contacts of TB infectious cases was associated with age and male sex but it is possible that genetic factors contribute to the susceptibility to *Mycobacterium tuberculosis* infection. However, the study did not look into, intensity of exposure to the case and/or severity of disease in the case.

This study found no association of tuberculin positivity and malnutrition, as well as no association with the bacteriological cases or cavity formation in chest x-ray. None of the children with positive TB contact cases had bacteriological proven TB.

Although TB may develop at any time in the course of the HIV infection[4] our study showed that 35.1% of under five children with suspected TB had CD4+ T lymphocytes <20% and 16.2% of children aged five years and above had CD4+ T lymphocytes <15%. It was also observed that 86.5% of children with TB were in HIV stage 3 or 4. Severe immunosuppression and advanced HIV disease were found to be significant risk factors for TB. This is in consistency with a study from Côte d'Ivoire in which the risk of TB was four times higher in children with a CD4+ T lymphocyte percentage of below 15% determined.[42] This can be explained by a fall in cell mediated immunity which eventually makes a host susceptible to develop TB as it depends on competent cellular immunity. Due to descriptive nature of the study, it is not clear whether severe immunosuppression and or advanced HIV disease led to development of TB or vice versa. There was no correlation between positive TST and CD4+ T-lymphocytes.
Results obtained from this study also agrees with other studies on clinical presentation and outcome of TB in a cohort of HIV- infected children on anti-retroviral therapy, which found that there were significantly more children with severe immunosuppression among those diagnosed with TB compared to those without TB. [43, 44]

In children with good immunity as evidenced by high CD4+ T lymphocyte percentage and a low viral load, the manifestations of TB are essentially similar to those who are HIV negative. As immunity declines, there is a rising incidence of atypical presentations of TB, and diagnostic difficulties are posed by the rather non-specific clinical features which may be confused with those of other HIV related infections.

Findings from this study showed that children on ARVs were less likely to have TB as shown by 36.7% children with TB not on ARVs, and 87.7% of children without TB being on ARVs. ARV reduces the HIV viral load, which allows CD4+ T lymphocytes repletion and assists with the restoration of immunological function. This findings concur with a study

done previously in adults and in children to determine impact of antiretroviral therapy on the incidence of TB which showed that compared ARV to non-ARV-treated patient groups, TB incidence was higher in ARV-naive patients, respectively.[31,43-45] When multivariate analysis was done, TB incidence was statistically significantly lower in ARV-treated compared to ARV naïve patients.[31,43,44,45]

Radiological (chest x-rays) findings suggestive of TB in this study varied. Abnormalities commonly seen were features suggestive of pneumonia, infiltrates and hilar and or mediastinal lymphadenopathy, and other findings including lung collapse, miliary TB and consolidation. Less commonly seen were cavity formations. This is in consistency with findings from a study done at MMC on radiological evaluation of children with cough of more than two weeks with and without HIV infection, in which lung infiltration and hilar adenopathy were fairly common in HIV seropositive patients. [36]

Cavitations were seen in 2 underfive children, both of whom were females with no history of contact to sputum smear positive TB case, but both had advanced HIV disease. One child was 37 months, had two positive sputum smear results, and was on ARV with CD4+ T lymphocyte of 53% and HIV stage 4. She was showing an improvement to TB treatment at one month of initiation. The second child was six months, not yet on ARV and CD4+ T lymphocyte of 19%, but unfortunately she died in the first month of initiation of anti-TB. Cavitations are rare in young children but seen in adolescents, who may develop TB reactivation similar to that observed in adults. The ability to form cavities in children in our study did not appear to be directly related to the CD4+ T lymphocyte percentage, but both had advanced HIV disease. Occurrence of cavitation due to TB in children has been reported previously.[4] Cavitary lesions in children may arise by acute cavitation following extension of a recent primary focus and evacuation of its contents, *exogenous* reinfection from hematogenous spread, rapid lung destruction after bronchial spread and evacuation of caseous material from a slowly growing focus.[46] In this study, female children were more commonly affected, and this was also observed by Vijayasekaran et al.[46]

Previous studies showed radiographic abnormalities are caused by a combination of lung diseases and mechanical changes caused by airway obstruction resulting from enlarging intrathoracic nodes. Chest radiography findings from previous studies also vary [8,16,30,33,36,37,47] like in this study and includes segmental hyperinflation, atelectasis, alveolar consolidation, interstitial densities, and, rarely a focal mass. Importantly, an abnormal radiograph, and a history of exposure from an adult with open TB remains the most effective method for diagnosing TB in children. It is very well known that the sensitivity and specificity of symptoms and signs of TB in HIV-infected individuals are very low and can lead to either misdiagnosis particularly when radiographs and other diagnostic tests are not done. [8,24]

Study findings showed that positive sputum AFB smear microscopy was found in 2 (5.4%) children with TB. Of these two children, one was a female (37 months) mentioned above, and the other child was male (77 months) with no history of open TB contact, was in HIV disease stage 3 and CD4+ T lymphocytes of 3%, he was not yet on ARVs. This is in agreement with previous study findings that showed that bacteriological confirmation of TB using microscopy is often difficult in children, because of few AFB and difficulties in obtaining clinical samples. It was reported that sputum is smear-positive in ≤10–15% of those with probable TB. [8,23,24]

Sixteen children with severe immunosuppression were not on ARVs. Out of 16, eleven were found to have TB, one of whom died before anti-TB initiation, and one child died within two weeks of starting anti-TB. Five children who did not have TB were started on ARVs as per HIV and AIDS National Guideline. Of the remaining nine, ARVs were initiated within a month following recommendations from the national guideline.[38]
Nineteen cchildren with severe malnutrition were referred to the ward for children with severe malnutrition for further management.

Thirty six children with TB were started on combined (fixed dose) 4-drug regimen containing isoniazid, rifampicin, pyrazinamide and ethambutol as per Tanzanian NTLP guideline.[38] Cotrimoxazole prophylaxis was initiated for children who were not on this prophylaxis. Two children with positive TB contact but found not to have TB were referred to TB clinic for INH preventive treatment as per Tanzania NTLP guideline.[38] This is important because studies have found a missed opportunity for TB chemoprophylaxis, among such observation was noted during a review of 596 cases of culture-confirmed childhood TB in South Africa.[40]

For the one child who developed jaundice and hepatomegaly at the end of the third week, the child was admitted for further management including exclusion of hepatitis. The child continued with ARVs and Cotrimoxazole of which he had been using for sevn months pending results. This is important because the cause may be a drug induced adverse event which could result in longer treatment duration, with again an increased risk of drug interactions and poor adherence to either or both HIV and TB treatment.[38,48]

Study limitations included: Technical difficulties in collecting sputum especially in children below five years of age; mostly it was probable TB so this could have diagnostic errors thus follow-up of these children is recommended.

8. **Conclusions**: One-fifth of children had HIV-TB co-infection. HIV-positive children with four or more clinical manifestations, low CD4+ T-lymphocyte percentage and advanced HIV disease were more likely to have TB.

9. **Recommendations**: Combination of clinical manifestations, radiological findings and sputum examination still be used to diagnose TB in HIV-infected children. History of open TB contact should be enquired routinely whenever HIV-infected children attend health care facilities. Follow up study should be done in children with low CD4+ T-lymphocyte percentage and advanced HIV disease to decide on their use as additional criteria for TB suspicion and aid TB diagnosis in HIV-infected children.

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