ASSOCIATION BETWEEN FINGER CLUBBING AND CHRONIC LUNG DISEASE IN HIV INFECTED CHILDREN AT KENYATTA NATIONAL HOSPITAL

A DISSERTATION PRESENTED IN PART FULFILLMENT FOR THE DEGREE OF MASTERS OF MEDICINE IN PAEDIATRICS AND CHILD HEALTH (M Med Paediatrics), UNIVERSITY OF NAIROBI

BY

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DECLARATION

I hereby declare that this dissertation is my own and to the best of my knowledge it has not been presented for examination in any other institution.

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DEDICATION

To my late father Eng. Albert Lachawu Mashandich who inspired me to do medicine and postgraduate studies.

To my mother, Mrs Violet Yeko for her constant support and prayers.

To my husband Frank and children: Gloria, Amani and Jewel for their patience, constant encouragement and support throughout my postgraduate studies.

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LIST OF ABBREVIATIONS

- AIDS-Acquired Immunodeficiency Syndrome
- ART- Antiretroviral therapy
- CCC-comprehensive care clinic
- CD4- cluster of differentiation
- HIV- human immunodeficiency virus
- IRIS- immune reconstitution inflammatory syndrome
- KNH-Kenyatta National Hospital
- LIP- lymphoid interstitial pneumonitis
- TB- Tuberculosis

ABSTRACT

Background: Finger clubbing in HIV infected children is associated with pulmonary diseases like bronchiectasis, TB, and LIP. Pulmonary involvement is responsible for great morbidity and mortality in HIV infected children. Finger clubbing is a clinical sign that is easy and quick to detect without sophisticated equipment. Therefore, finger clubbing could provide simple screening tool to identify children for further evaluation for chronic lung disease and hence timely intervention which may result in lower morbidity and mortality.

Objective: To determine the association of finger clubbing and chronic lung diseases in HIV infected children. Secondary objective was to determine clinical correlates (in terms of WHO clinical staging, CD4 counts/ percentage, antiretroviral therapy duration and pulmonary hypertension) of finger clubbing among HIV infected children.

Methodology: Hospital based prospective case control study was conducted at KNH Paediatric wards and CCC. Cases were 60 HIV positive children with finger clubbing and controls were 60 HIV infected children without finger clubbing. A total of 120 HIV infected children upto 18 years whose parents gave consent were recruited into the study between February 2012 and January 2013. Baseline characteristics and physical examination, laboratory tests, chest radiographs and echocardiography were undertaken and recorded in questionnaires. Diagnosis of presence or absence of chronic lung disease was then made. Data was recorded daily in questionnaires by the investigator. The obtained data was entered into the Statistical Package Social Sciences (SPSS) data entry programme and analyzed using SPSS/PC+ version 9 programme. The data was then summarized in frequency tables. The differences between cases and controls were determined using Chi square test for categorical variables.

Continuous variables was analyzed and presented as medians with interquartile ranges (IQR) then compared between cases and controls using Mann Whitney U test. Odds ratios were calculated for categorical data to estimate the magnitude of risk among the cases. All the statistical tests were performed at 95% confidence interval (5% level of significance).

Results: Diagnosis of chronic lung disease was six times more common among the finger clubbed, 33 (55%) than the non finger clubbed patient, 10 (16.7%), OR 6.1 [95% CI 2.6-14.3], p<0.001**.** Finger clubbed patients had 2.6 times risk of being diagnosed with hypoxemia, 28 (46.7%), OR 2.6 (95% CI 1.2-5.7), p=0.013, and 4.4 times risk of pulmonary hypertension, 28 (46.7%), OR 4.4 (95% CI 1.9-10.2), p=0.001 as compared to the controls. Those with finger clubbing had advanced disease in WHO stage III/ IV (91.7%) compared to non finger clubbed patients (68.3%), OR 6.4 [95% CI 2.0-20.2], p<0.001. Patients with finger clubbing had lower CD4 cells count and percent (median 369cells, 13%) compared to non clubbed patients (median 861cells, 28%), p<0.001. Duration of ART use was shorter in the finger clubbed patients (5.5 months) compared to non finger clubbed patients (median 40 months) p<0.001.

Conclusion: The diagnosis of chronic lung disease was more common in patients with finger clubbing than those without it and the presence of finger clubbing in HIV infected children was associated with advanced WHO stage III or IV, lower CD4 counts and percentage and shorter duration of ART use. There is a higher risk of developing pulmonary hypertension in HIV infected children with finger clubbing.

Recommendations: All HIV infected children should be examined for the presence of finger clubbing and those found with it should have pulse oximetry, chest radiolograph and echocardiography to assess for the presence of chronic lung disease and its complications.

1.0 INTRODUCTION

Definition of finger clubbing

Finger clubbing is alternatively called Hippocratic fingers, watch-glass nails, or drumstick fingers (1).

It is characterized by the enlargement of the terminal segments of the fingers and/or toes that result from the proliferation of the connective tissue between the nail matrix and the distal phalanx. Although most often symmetrical, clubbing can be unilateral or even unidigital (2).

It may also be described as a focal, bulbous enlargement of connective tissue on the dorsal surface of the terminal phalanges of the fingers and/or toes (3).

Finger clubbing observed in HIV infected patients could be attributed to pulmonary or nonpulmonary conditions. Respiratory complications in HIV-infected children are common and responsible for substantial morbidity and mortality (4-7). The respiratory complications can be acute or chronic HIV associated respiratory diseases. The spectrum of chronic HIV associated respiratory illnesses include lymphoid interstitial pneumonitis (LIP), tuberculosis (TB), chronic infections, bronchiectasis, interstitial pneumonitis, immune reconstitution inflammatory syndrome (IRIS) and malignancies.

Finger clubbing has been associated with a variety of pulmonary diseases like bronchiectasis, TB, lung abscess and lymphoid interstitial pneumonitis.

Nonpulmonary conditions accompanied by finger clubbing include cyanotic heart diseases, infective endocarditis, inflammatory bowel disease and liver cirrhosis and malignancies.

Finger clubbing may also occur as an idiopathic finding.

Aetiology

The association of clubbing with a number of infectious, neoplastic, inflammatory, and vascular diseases has been known by clinicians since Hippocrates first described clubbing in a patient with empyema in the fifth century BC(2). Finger clubbing has been associated with various underlying pulmonary, cardiovascular, neoplastic, infectious, hepatobiliary, mediastinal, endocrine, and gastrointestinal diseases. Finger clubbing also may occur, without evident underlying disease, as an idiopathic form or as a Mendelian dominant trait (8).

Pathogenesis

One of the mechanism by which digital clubbing occurs is when platelet precursors fail to fragment into platelets within the pulmonary circulation. The fragments are large enough to lodge in the vascular beds of the fingertips, and, subsequently, they release platelet-derived growth factor and vascular endothelial growth factor. These factors have been shown to have general growth-promoting activity and causes increased capillary permeability and connective tissue hypertrophy which finally leads to finger clubbing (9-11).

In hypoxic conditions with extra pulmonary shunting of blood, large megakaryocyte fragments fail to enter the pulmonary circulation. Instead they gain access to the systemic circulation impacting at the most distal sites, there releasing growth factors and thus inducing clubbing. Vascular endothelial growth factor also plays a central role in development of digital clubbing by producing vascular hyperplasia, edema, and fibroblast/osteoblast proliferation. Vascular endothelial growth factor is a platelet-derived factor induced by hypoxia, and it is also abnormally produced by diverse malignant tumors fostering their uncontrolled growth (12).

Hypoxia has also been proposed as an alternative explanation for clubbing in pulmonary diseases. An increase in hypoxia may activate local vasodilators, consequently increasing blood flow to the distal portion of the digits. However, in most cases, hypoxia is absent in the presence of clubbing, and many diseases with notable hypoxia are not associated with clubbing. Proposed vasodilatory factors include ferritin, prostaglandins, bradykinin, adenine nucleotides, and 5-hydroxytryptamine (13).

Pathophysiology

Clubbing occurs in stages; first, there is a periungual erythema and a softening of nail bed, giving a spongy sensation on palpation, followed by an increase in the normal 160 degrees angle between the nail bed and the proximal nail fold. This increased angle causes the nail to develop a convexity as it grows. Eventually the nail and periungual skin appear shiny and the nail develops longitudinal ridging. The depth of the distal phalanges increases and the distal interphalangeal joints may become hyperextensible. Clubbing usually develops over years but in certain conditions may develop subacutely (14).

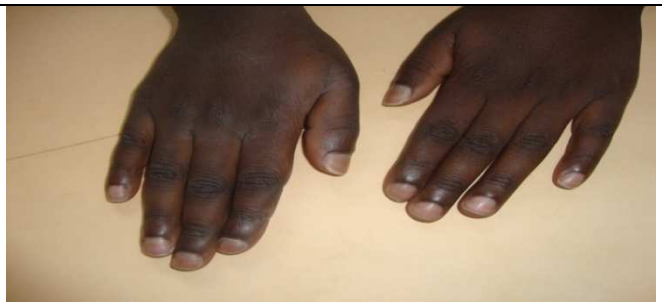
Clubbing is graded from grade one to four (15) as shown in figure 1.

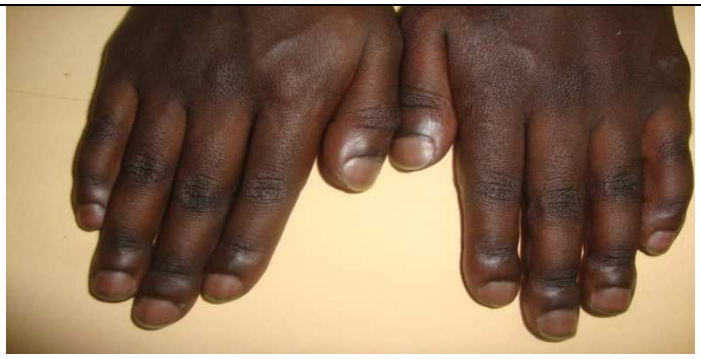
GRADE I: Fluctuation and softening of nail bed

GRADE II: Obliteration of the angle of the nail bed

GRADE III: swelling of the subcutaneous tissues over the base of the nail causing the overlying skin to become tense, shiny and wet and increasing the curvature of the nail, resulting in parrot beak or drumstick appearance.

Grade IV**:** Swelling of the fingers in all dimensions associated with hypertrophic pulmonary osteoarthropathy causing pain and swelling of the hand, wrist etc, and radiographic evidence of subperiosteal new bone formation.





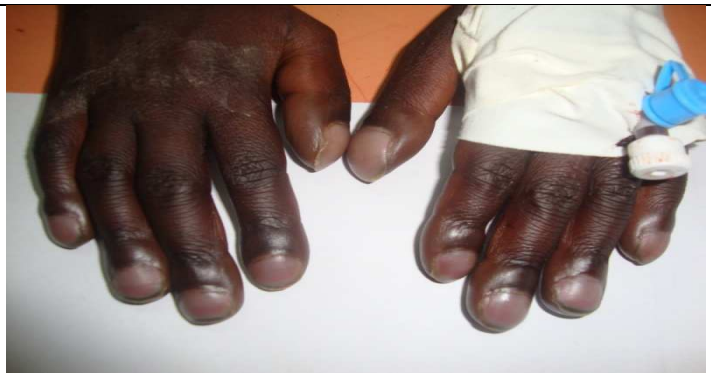


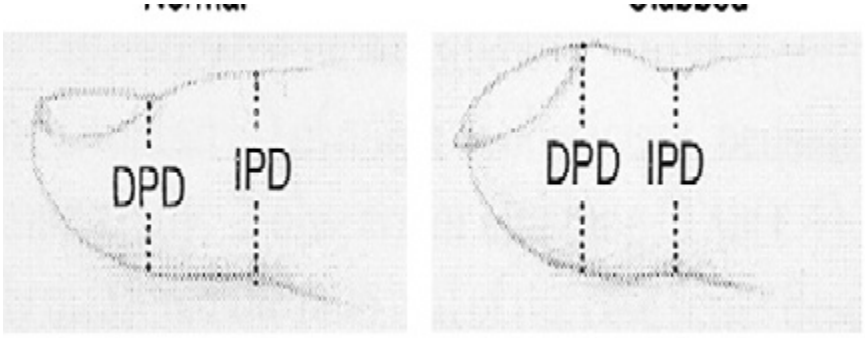
Figure 1: Finger clubbing.

Histologically, the main features observed are a dilatation and thickening of the small blood vessel walls (16) as well as numerous arteriovenous anastomoses in the nail beds of the clubbed fingers (17).

Diagnosis

Diagnosis of finger clubbing is confirmed by determining phalangeal depth ratio and with the presence of Schamroth sign (2). Phalangeal depth ratio is the ratio of distal phalangeal diameter (DPD) over interphalangeal joint diameter (IPD) of the index finger as shown in figure 2. Phalangeal depth ratio of more than one indicates clubbing. This is measured using vernier calipers or more precisely, finger casts.

NORMAL CLUBBING



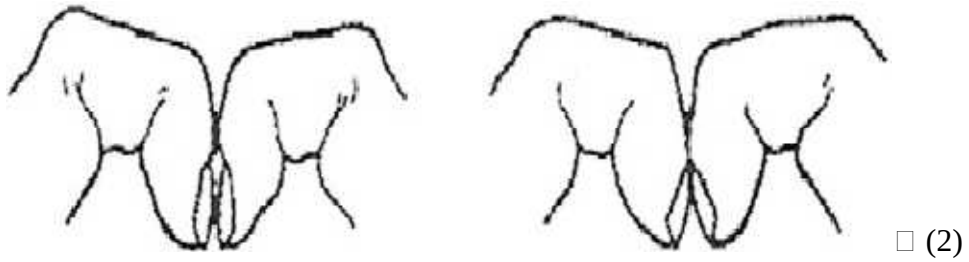
Adopted from (2)

Figure 2: Demonstrating Phalangeal depth ratio.

Clubbing is diagnosed when DPD/IPD ratio > 1

Schamroth sign refers to when the diamond-shaped window is absent when dorsal surface of terminal phalanges of similar fingers are opposed together and a prominent distal angle forms between the ends of the nails (Figure 3).

NORMAL CLUBBING



(2)

A B

Figure 3: Schamroth's sign in clubbing**.**
Normal (A) and obliterated (B) Schamroth's sign in Clubbing (11)

2.0 LITERATURE REVIEW

ASSOCIATION OF FINGER CLUBBING AND LUNG DISEASES

The association of clubbing with pulmonary disease factors, such as chronic inflammation or hypoxemia, is frequently recognized clinically. In a study, Paton et al (18) showed that digital clubbing is associated with pulmonary function abnormalities particularly hypoxemia and abnormal forced expiratory volume in 1 sec (FEV1) in patients with a wide spectrum of respiratory diseases. Pitts-Tucker et al (19) also reported on finger clubbing in 103 patients with cystic fibrosis. They found significant correlations between finger clubbing and percent predicted forced vital capacity (FVC), and percent predicted forced expiratory volume in 1 second (FEV).

The presence of clubbing in HIV-infected patients is attributed to concomitant pulmonary infections which result in hypoxia. Respiratory disease is the major cause of morbidity and mortality in HIV-infected children in developed and developing countries (4).

Bacterial pneumonias can be recurrent in AIDS patients and are the main cause of hospital admissions. The bacteria that cause pneumonia in immune competent children are the same ones that cause pneumonia in AIDS patients. HIV positive children are at higher risk for pneumonia associated to opportunistic agents, like protozoa, viruses, fungi, and mycobacteria. Pneumocystis jirovecii pneumonia and lymphocytic interstitial pneumonitis (LIP) used to be even more prevalent until very recently (20).

CHRONIC LUNG DISEASE IN HIV-INFECTED CHILDREN

Chronic lung disease is common in HIV-infected children with increasing age (21, 22). A longitudinal birth cohort study reported a cumulative incidence of chronic radiographic lung changes in HIV-infected children of 33% by 4 years of age (21). The commonest chronic radiological changes were increased bronchovascular markings, reticular densities or bronchiectasis (21, 22). Chronic changes were associated with lower CD4 cell counts and higher viral loads; radiological resolution of these may reflect declining immunity (21).

Chronic cough and chronic lung disease are also known to be one of the major clinical presentations of HIV/AIDS (23, 24).

The spectrum of HIV-associated chronic lung disease includes lymphocytic interstitial pneumonia, chronic infections, immune reconstitution inflammatory syndrome (IRIS), bronchiectasis, malignancies like Non-Hodgkin's lymphoma and Kaposi's sarcoma, bronchiolitis obliterans and interstitial pneumonitis. In HIV and TB high prevalence areas, TB is a common cause of chronic lung disease (23-6).

Lymphoid interstitial pneumonia is an AIDS defining condition which has a better prognosis than other defining illnesses. Children with lymphocytic interstitial pneumonia usually present after 2 years of age, and associated clinical features include generalized lymphadenopathy, bilateral nontender parotid enlargement, mild hypoxemia and marked hepatomegaly. Secondary bacterial disease due to S. pneumonia or Salmonella is common. Children may survive for years with a course characterized by recurrent episodes of acute lower respiratory tract infections. Cor pulmonale or bronchiectasis may develop as a complication. Chest radiographs often show a diffuse reticulonodular pattern, more pronounced centrally which may be difficult to distinguish from pulmonary or miliary TB. The definitive diagnosis of lymphocytic interstitial pneumonia is only made by biopsy of the

lung. In early stages of lymphocytic interstitial pneumonia, before chronic irreversible lung lesions develop, prompt antiretroviral therapy can reverse lymphocytic interstitial pneumonia. Similarly, recurrence of lymphocytic interstitial pneumonia occurs in the setting of HIV treatment failure. Corticosteroids are useful in alleviating symptoms (4, 25-6).

HIV-positive children are at risk of diagnostic error and delayed diagnosis of TB because of overlapping clinical and radiographic features with other lung diseases. The diagnosis of TB in children with HIV is usually complicated by several factors which include non-specific clinical findings, frequent involvement of extra-pulmonary sites, coexistence of previously abnormal chest X-rays and often negative tuberculosis skin test (Mantoux test). Acute pneumonias and chronic lung diseases such as bronchiectasis and lymphocytic interstitial pneumonitis are difficult to distinguish from TB. TB presents with chronic cough, weight loss or failure to thrive and fever. Chest radiograph may show lymph node enlargement, airway compression, focal or diffuse consolidation, miliary pattern or cavity. Diagnosis can be made from history of TB contact, positive tuberculin skin test, chest X-ray, smear microscopy, and culture of specimens. Due to the multiple clinical manifestations of TB, often with non-specific signs and symptoms, it should always be included in the differential diagnosis of any child with HIV and lung disease. TB treatment consists of TB medications and atimes corticosteroids for airway compression. Complications of TB include fibrosis, bronchiectasis, and chronic atelectasis (25-7).

Chronic lung disease may result from recurrent or persistent pneumonia due to bacterial, mycobacterial, viral, fungal or mixed infections. Streptococcal pneumonia is the most common cause of bacterial pneumonia in HIV-infected children. Staphylococcal pneumonia also occurs and may be complicated by the development of an emphysema, pneumatocoele or lung abscess. Other causes of community acquired bacterial pneumonia in HIV infected children include gram negative pathogens such as klebsiella pneumonia, pseudomonas aeruginosa, hemophilus influenzae, non-typhoid salmonella and E. coli. Severe, destructive, persistent or recurrent bacterial pneumonia may lead to chronic lung disease such as bronchiectasis. Bacterial pneumonia presents with rapid onset, high fever, tachypnoea, and lower chest indrawing. Lobar consolidation, diffuse infiltrates on chest X-ray and blood culture help in the diagnosis. Treatment is with broad spectrum antibiotics and other supportive measures like oxygen administration (25).

Bronchiectasis may occur secondary to chronic infection including mycobacterium tuberculosis, or following recurrent bacterial infections, or after a severe viral lower respiratory tract infection or as a consequence of lymphocytic interstitial pneumonia. Bronchiectasis is defined as ‘‘irreversible dilatation of peripheral airways,’’ and is usually diagnostically established radiologically by chest radiography and chest high resolution computerized tomography scans. It usually presents with chronic cough productive of purulent sputum, halitosis and abnormal auscultatory chest findings. Bronchiectasis is managed by physiotherapy, antibiotics for intercurrent infections, surgery for localized disease and antiretroviral therapy. Bronchiectasis may lead to development of cor pulmonale (25, 28-30).

Immune reconstitution syndrome (IRIS) may occur within six weeks to several months after initiation of antiretroviral therapy. It results either from unrecognized mycobacterial infection or from a florid immune response directed against a mycobacterial antigen in those already on therapy for mycobacterial infection. IRIS is characterized by a seemingly paradoxical worsening in signs with increasing lymphadenopathy, new clinical, and radiological respiratory signs, and fever (25).

FINGER CLUBBING AND HIV INFECTION

Graham et al (31) studied a total of fifty two children with finger clubbing. They observed that digital clubbing in fifty two Malawian children aged between 4months - 12 years was associated with chronic lung disease and HIV infection, presenting as early as infancy. A history of persistent cough for at least 1 month (73%), tachypnoea (63%), and auscultatory abnormalities (65%) were common. Thirty one of these children were tested and 84% of them were HIV-antibody positive .This study recommended using the presence of clubbing as a diagnostic clue to childhood HIV infection in areas with a high prevalence of the disease.

Zar et al (32) studied a total of one hundred and fifty HIV infected children hospitalized with acute pneumonia in South Africa. This study found that finger clubbing occurred in 20% of HIV-infected children compared with 1% HIV-negative control patients. Clubbing was associated with lower pulse and respiratory rates, enlarged parotid glands and lower CD4 counts. The children with finger clubbing had radiological changes of lymphoid interstitial

pneumonia and had decreased in-patient mortality compared with the controls. In this study, it was concluded that in geographical areas with high HIV seroprevalence rates, the presence of clubbing in a child hospitalized for respiratory disease should raise the suspicion of HIV infection.

Dever et al (33) in an observational study of seventy eight HIV infected adults in New Jersey found finger clubbing in 36% (twenty eight) of HIV infected adult patients. Clubbed patients did not differ from nonclubbed patients with respect to most patient characteristics such as CD4 cell counts, HIV viral load and chest radiographic features. However, this study suggests that digital clubbing may serve as a clue to underlying HIV infection and therefore, HIV should be considered in the differential diagnosis of unexplained acquired clubbing.

Akolo C et al (23) undertook a study to determine the commonest symptoms and signs of HIV/AIDS in adults at presentation in Nigeria. They found that finger clubbing was one of the major signs of HIV/AID accounting for 8.5% of the signs in the 200 adults studied.

Symth A et al (34) in Zambia, however, demonstrated that finger clubbing is a poor sign of HIV infection in children. Forty seven children were studied and twenty one percent were found to be HIV-positive. Finger clubbing was present in only one child who was HIV-positive and had a persistent right-sided empyema requiring open chest drainage.

Cribier B et al (35) undertook a prospective controlled study in adults on nail changes in HIV infected adults. A total of 155 HIV-1 positive patients and 103 healthy HIV-negative control subjects of comparable age and sex ratio were included. The study by Cribier B et al (35) found that nail changes occurred in 68% of those infected with HIV compared to 34% of the controls. Finger clubbing accounted for 6% of the nail changes in the HIV infected patients. The conclusion of the study was that nail symptoms are much more frequent in patients with HIV than in healthy controls.

A study was done in Uganda on two hundred adult patients with pulmonary TB (36). Eighty two percent of them were HIV positive. The study found no association in HIV infection and clubbing in patients with pulmonary TB. The proportion of patients with TB who had digital clubbing was comparable between HIV-infected and HIV-uninfected patients. However, this study did not assess the frequency of clubbing in HIV-positive patients without TB.

3.0 STUDY JUSTIFICATION AND UTILITY

It is hypothesized that presence of finger clubbing in HIV infection may indicate underlying lung pathology. Respiratory conditions in HIV infected children are responsible for substantial morbidity and mortality. Finger clubbing is a clinical sign that is easy and quick to detect without sophisticated equipment and very feasible to diagnose. Therefore, finger clubbing could provide simple screening tool to identify children for further evaluation for chronic lung disease and hence timely intervention which may result in lower morbidity and mortality. The aim of this study is to evaluate association and clinical correlates of finger clubbing in HIV infected children in relationship to chronic lung conditions and other factors like WHO staging, CD4 counts/ percentage, ART duration and pulmonary hypertension.

4.0 RESEARCH QUESTION AND STUDY OBJECTIVES

4.1 Research question

What is the association of finger clubbing and chronic lung diseases among HIV infected children?

4.2 STUDY OBJECTIVES

4.2.1 Primary Objective

To determine the association between finger clubbing and chronic lung disease among HIV infected children.

4.2.2 Secondary Objectives

To define how WHO staging, CD4 counts/ percentage, ART duration and pulmonary hypertension correlates with finger clubbing among HIV infected children.

5.0 STUDY METHODOLOGY

5.1 Study design

This was a case control study. The cases were defined as HIV-positive children with finger clubbing present. The controls on the other hand were HIV-positive children without finger clubbing.

5.2 Study area

The study was conducted at the Kenyatta National Hospital (KNH) comprehensive care clinic (CCC) for HIV infected children and paediatric general wards. KNH is situated in Nairobi, the capital city of Kenya, and is the largest referral hospital in East and Central Africa.

Both the adult and paediatric HIV clinics were co-located in the same building but operated separately. Most of the patients who attended the clinic were from the city and its environs although some were referred from other parts of the country. The pediatric clinic operated on weekdays from 8am to 5pm and 20 to 40 patients are seen per day. Upon arrival at the clinic, patients report to the records office where their record files were retrieved from storage and the patient registered in a clinic attendance register by the records clerks. Generally, the clinicians saw the patients in the order of registration with exception of very sick children who were triaged and seen without waiting, or those delayed due to other unusual reasons such as; a lost file or pending laboratory results. Once seen by a clinician those requiring laboratory investigations are sent to the laboratory for sample collection. Stable patients were reviewed with the results during the next appointment.

The paediatric general wards were four located on third floor. They catered for children birth to thirteen years. Some of the patients were referred from other facilities in different parts of Kenya and environs of Nairobi. Children with different medical and oncology conditions were admitted to those wards. The wards were one of the entry points of the newly diagnosed HIV-positive children into care. Other children could also be admitted directly through the comprehensive care clinic.

5.3 Study Population

The study population was derived from paediatric patients admitted at the pediatric general wards and those attending the pediatric HIV clinic at Kenyatta National Hospital Comprehensive Care Center. Over 1100 children of age birth to fourteen years attended the clinic regularly and of those 650 were on antiretroviral therapy. A few adolescent patients

between the age of 14 and 17 years who preferred to continue visiting the paediatric clinic were allowed to continue care at the paediatric clinic.

5.4 Sample Selection

5.4.1 Inclusion criteria

- HIV infected children and adolescents aged eighteen years and below.
- Children whose parents/guardians consented to participate in the study
- HIV infected children on ART or ART naive.

5.4.2 Exclusion criteria

- Those diagnosed with HIV who did not consent and those that had conditions other than of the lung known to cause finger clubbing which included:
  i. Patients with cardiac diseases or on examination found to have a congenital heart disease or rheumatic heart disease.
  ii. Patients who had liver pathology like hepatitis, cirrhosis or abnormal liver function tests with the liver enzyme levels five times above the upper limit.
  iii. Patients who had any form of malignancy or on treatment for malignancy
  iv. Patients with chronic diarrhea of more than fourteen days.

5.5 Sample size estimation

In this study, two proportions from STATA version 9.0 (College Station Texas) was used. It is a two–sample comparison of proportions. It is software which is pre-programmed and gives sample size estimation once power, alpha and proportions are fed on the computer.

Power $(1-\beta) = 0.8$

Alpha $(\alpha) = 0.05$

Ratio of Cases: Controls = 1:1

P1 = 0.6 (proportion with chronic lung disease among children with finger clubbing as per Malawian study by Graham et al, (31).

P2 =0.3(proportion with chronic lung disease in HIV infected children by Norton et al study, (21).

Cases: 60 HIV infected children who had finger clubbing.

Controls: 60 HIV infected children without finger clubbing.

This gave a minimum sample size of 120

5.6 Sampling method and procedure.

The sampling and procedure of the study was done as shown in Figure 4 below.

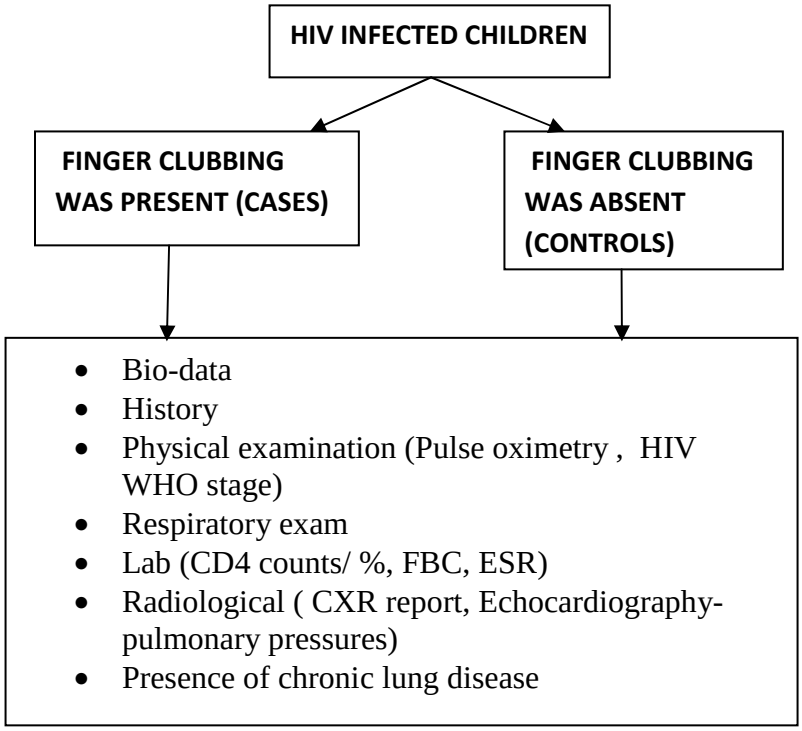


Figure 4: Flow chart of study procedure

5.6.1 Study Procedure

1. The investigator visited the study areas daily on weekdays between 9.00am and 4.00pm. The children were screened with the help of research assistants who had prior training by the principle investigator on how to identify finger clubbing

2. The study was explained to parents and older children. Informed consent was obtained from parents or guardians and assent from older children by the principle investigator. Those children that satisfied the inclusion criteria with finger clubbing were recruited as cases. In this study, finger clubbing was defined as finger clubbing that is obvious to the investigator's eye, and then by determination of present schamroth's sign.

In this study, schamroth's sign was present if the dorsal surfaces of the terminal phalanges of the opposite fingers were placed together and there was disappearance of the normal diamond shaped window at the bases of the nail beds.

Finger clubbing was graded by principle investigator as grade I, II, III or IV in all participants recruited as cases.

3. To select controls, research assistants identified HIV infected children who did not have finger clubbing and forwarded them to the investigator. The investigator then explained the study to the parents and older children. Those children were then screened by the principle investigator to ascertain that they had no obvious finger clubbing and absent schamroth's sign and were then recruited as controls.

4. Information on demographic characteristics, previous respiratory illness and current chest symptoms were obtained by the investigator through interviewing the parents or guardians of all recruited children (both cases and controls). History on the duration of the current illness and other serious intercurrent illness other than the lungs were also recorded. History of antiretroviral use and duration was elicited and these were recorded in a pretested questionnaire (Appendix 2).

5. Physical examination including pulse oximetry and WHO clinical staging at recruitment (Appendix 4 and 5) were done on each child recruited as a case or control by the principle investigator. The information was also recorded in the pretested questionnaire.

Hypoxia was defined as oxygen saturations less than 92% on pulse oximetry (37).

6. All the recruited patients were then sent to the laboratory for CD4 counts (Appendix 6 and 7) and full blood counts and erythrocyte sedimentation rate. The patients were then scheduled for chest radiograph and echocardiogram within one week of enrolment to the study.

7. Chest radiographs obtained from all participants were reported by first radiologist, and then a second independent radiologist also reported on it as shown in Appendix 8.

8. The diagnosis of chronic lung disease if present was made on both cases and controls based on the investigator's clinical examination and radiological findings of the patients.

5.7 STUDY DEFINITIONS/DIAGNOSIS

Chronic lung disease was defined as (modified as per Zar study, 25):

1. Presence of chronic cough for more than a month together with
2. Chest radiological abnormalities plus
3. Presence any of the following signs:
    - Fever of more than 37.5^0 C
    - Chest deformity which included pes carinatum, pes excavatum, hyperinflated chest.
    - Abnormal breath sounds like wheeze, crackles or bronchial breathing

The diagnosis of chronic lung disease included the following conditions: tuberculosis, lymphocytic interstitial pneumonia, bronchiectasis, and chronic chest infections.

Tuberculosis was diagnosed according to the Kenya National Paediatric Tuberculosis Care Guidelines (38) which follows the following algorithm:

Presence of two or more of the following symptoms

- cough for more than two weeks,
- weight loss or poor weight gain,
- persistent fevers and /or night sweats for more than two weeks,
- fatigue, reduced playfulness, less active

Plus presence of two or more or more of the following:

- Positive contact history,
- Respiratory signs,
- Chest radiography suggestive of pulmonary tuberculosis
- Positive montoux test where available.

Bronchiectasis was diagnosed from both the clinical presentation of halitosis, stunting and copious production of infected sputum and suggestive chest radiograph findings (25).

Presumptive diagnosis of **lymphoid interstitial pneumonitis (LIP)** was made on the basis of both symptoms and chest radiographic findings. The symptoms included cough and mild tachpnoea with any of following: generalized lymphadenopathy, bilateral non-tender parotid enlargement and hepatomegaly or spleenomegaly. Presence of chest radiographic changes of reticulonodular shadowing and non-response to anti-microbial therapy was suggestive of LIP (25).

8. Echocardiography to determine the pulmonary pressures was performed on all recruited study patients by a paediatric cardiologist assisted by the principle investigator. Standard two dimension and colour flow Doppler was done to asses and rule out congenital and rheumatic heart disease.

Trans-thoracic echocardiography was performed in all patients using of a portable Vivid I Echo colour ultrasound System® echocardiogram machine. Cardiac measurements were performed according to the guidelines of The American Society of Echocardiography. Colour codes guided spectral Doppler was sampled at the tricuspid valve in the short axis and apical four chamber view. A minimum of five sequential complexes were recorded. Continuous wave Doppler sampling of the peak tricuspid regurgitant jet velocity was used to estimate the pressure gradient between the right ventricular and the right atrium using the modified Bernoulli's equation (Gradient =4× [TRV²]) where TRV means tricuspid regurgitation velocity.

The pulmonary arterial systolic pressure was calculated as the sum of the pressure gradient (Gradient =4× [TRV²]) and a constant assumed right atrial pressure of 10mmHg. This calculation was pre-programmed in the echocardiography machine used in the study.

Trace or absence of tricuspid regurgitation was indicative of normal pressure.

Pulmonary arterial hypertension was defined as tricuspid regurgitation velocity (TRV) of 2**.**5m/s and above equivalent to right ventricular to right atrial pressure gradient of 25mmHg (as derived using the modified Bernoulli's equation) and pulmonary arterial systolic pressure of 35mmHg.

For the purpose of this study, pulmonary arterial hypertension was graded as mild if pulmonary arterial systolic pressure of 35-49mmHg, moderate if pulmonary arterial systolic pressure of 50-70mmHg, and severe if pulmonary arterial systolic pressure was greater than 70mmHg.

9. Both cases and controls were recruited by consecutive sampling whereby every patient who satisfied the inclusion criteria was serially recruited until the desired sample size was achieved. In this study, matching of the cases to the controls was not done.

5.8 Study tool

A questionnaire was used to collect data. Sample questionnaire is shown in Appendix 2.

5.9 Study duration

The study was carried out between February 2012 and January 2013.

6.0 DATA ANALYSIS AND MANAGEMENT OF DATA

Data was recorded daily in questionnaires by the investigator. The obtained data was entered into the Statistical Package Social Sciences (SPSS) data entry programme and analyzed using SPSS/PC+ version 9 programme. The data was then summarized in frequency tables. The differences between cases and controls was determined using Chi square test for categorical variables such as sex, previous treatment of lung disease, oxygen saturations, pulmonary pressure and use of antiretroviral therapy.

Continuous variables such as age, CD4 counts, CD4% and duration of ART was analyzed and presented as medians with interquartile ranges (IQR) then compared between cases and controls using Mann Whitney U test. Odds ratios were calculated for categorical data to estimate the magnitude of risk among the cases. All the statistical tests were performed at 95% confidence interval (5% level of significance).

Results are presented in form of tables, bar graphs and frequency tables.

7.0 ETHICAL CONSIDERATIONS:

1. The study was undertaken after written approval by the Department of Paediatrics and Child Health, University of Nairobi, and the Ethical Review Committee, Kenyatta National Hospital/University of Nairobi.
2. The purpose of the Study was explained to the Children's Parents or Guardians and a Written Consent and assent was obtained prior to enrolling any child in the study.
3. Strict Confidentiality was observed throughout the entire study period. The study participants were given study identification numbers and study data was only accessible to the investigators.
4. Clinically important findings and laboratory results were made available to the primary ward doctor or clinic teams to guide patient care.
5. The study findings were presented to the University of Nairobi (UON) Department of Paediatrics and Child Health Academic Staff and Students.

8.0 RESULTS

8.1 Introduction to results

The total number of participants recruited into the study was 120 HIV infected children at KNH pediatric wards and comprehensive care clinic during the period of February 2012 to January 2013. The study participants comprised of 60 children with finger clubbing (cases) and 60 children without finger clubbing (controls).

8.2 Basic patients' demographic characteristics

There were 68 (56.7%) males in the study yielding a male-to-female ratio of 1: 1.3. Among the cases, there were 35 (58.3%) males and 25 (41.7%) females compared to controls where males were 33 (55%) and females 27(45%) (Table 2).The median age of children who participated in the study was 7.5 years (IQR 3.0 to 11.0). The cases were younger with a median age of 6.5 years (IQR 2.0-10.5) compared to 9.0 years (IQR 4.5-13.5) for the controls (Table 1).

Cases and controls were not significantly different in relation to their age, sex and previous treatment of lung disease. However, significantly less cases, 22 (36.7%) were recruited from the comprehensive care clinic as compared to controls whose majority, 53 (88.3%) were recruited from the comprehensive care clinic, OR 0.1 [95% CI 0.0-0.2], $p<0.001$(Table 1).

Table 1: Baseline characteristics

Characteristics	Overall (n=120)	Case (n=60)	Control (n=60)	OR (95% CI)	P value
Median age in years (IQR)	7.5(3.0-11.0)	6.5 (2.0-10.5)	9.0 (4.5-13.5)	-	0.083
Age group					
0-11 months	11 (9.2)	6 (10.0)	5 (8.3)		0.377
1-5 years	32 (26.7)	19 (31.7)	13 (21.7)		0.099
6-10 years	39 (32.5)	20 (33.3)	19 (31.7)		0.299
11-18 years	38 (31.7)	15 (25.0)	23 (38.3)		
Residence					
Nairobi	98(81.7)	46(76.7)	52(86.7)	0.5(0.2-1.3)	0.157
Outside Nairobi	22(18.3)	14(23.3)	8(13.3)		
Sex					
Male	68 (56.7)	35 (58.3)	33 (55.0)	1.1 (0.6-2.4)	0.713
Female	52 (43.3)	25 (41.7)	27 (45.0)	1.0	
Site					
CCC	75 (62.5)	22 (36.7)	53 (88.3)	0.1 (0.0-0.2)	<0.001
Ward	45 (37.5)	38 (63.3)	7 (11.7)	1.0	
Previous treatment of lung disease					
Yes	67 (56.3)	34 (56.7)	33 (55.9)	1.0 (0.5-2.1)	0.936
No	52 (43.7)	26 (43.3)	26 (44.1)	1.0	

8.3 The degree of finger clubbing.

Finger clubbing was graded from grade I to IV as shown below with majority of cases being Grade II, 35 (58.3%) [Figure 5].

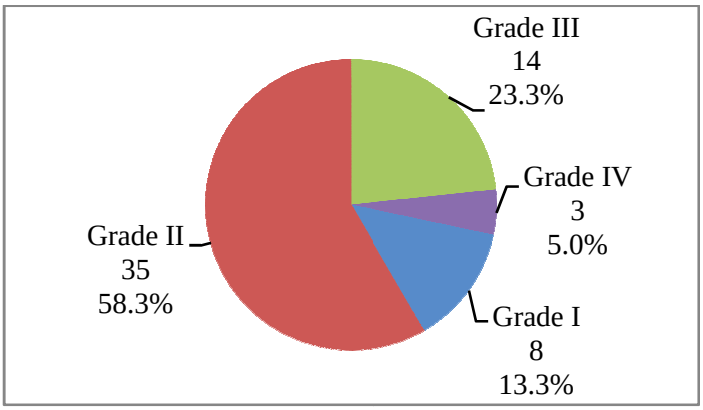


Figure 5: Grade of finger clubbing

8.4. Chest radiograph abnormalities.

The observed radiographic findings included patchy infiltrates, opacities, hilar adenopathy, lung consolidaton, cavitations, pleural effusion, lung collapse, reticulonodular shadowing and military nodules typical of tuberculosis. Abnormalities of the chest radiographs were reported in 90 (75%) of all study participants. Patients with finger clubbing were six times more likely to have abnormal radiographs compared to those without finger clubbing, OR 6.0(95% CI 2.2-16.1),p<0.001 as shown in table 2.

Table 2: Chronic lung disease and Chest radiography report.

Variable	**Overall n=120**	**Case n=60**	**Control n=60**	**OR (95% CI)**	**P value**
Chest x-ray report					
Abnormal	90 (75.0)	54 (90.0)	36 (60.0)	6.0 (2.2-16.1)	<0.001
Normal	30 (25.0)	6 (10.0)	24 (40.0)	1.0	
Presence of chronic Lung Disease					
Yes	43 (35.8)	33 (55.0)	10 (16.7)	6.1 (2.6-14.3)	<0.001
No	77 (64.2)	27 (45.0)	50 (83.3)	1.0	

8.5 Diagnosis of Chronic lung disease.

Overall, 43 (35.8%) of the patients had chronic lung disease as defined by the presence of chronic cough for more than a month and any of the following: fever, chest deformity, wheeze, crackles and/or bronchial breathing together with radiological changes. Diagnosis of chronic lung disease was six times more common among the cases, 33 (55%) than the controls, 10 (16.7%), OR 6.1 [95% CI 2.6-14.3], p<0.001 as shown in Figure 6 and Table 2.

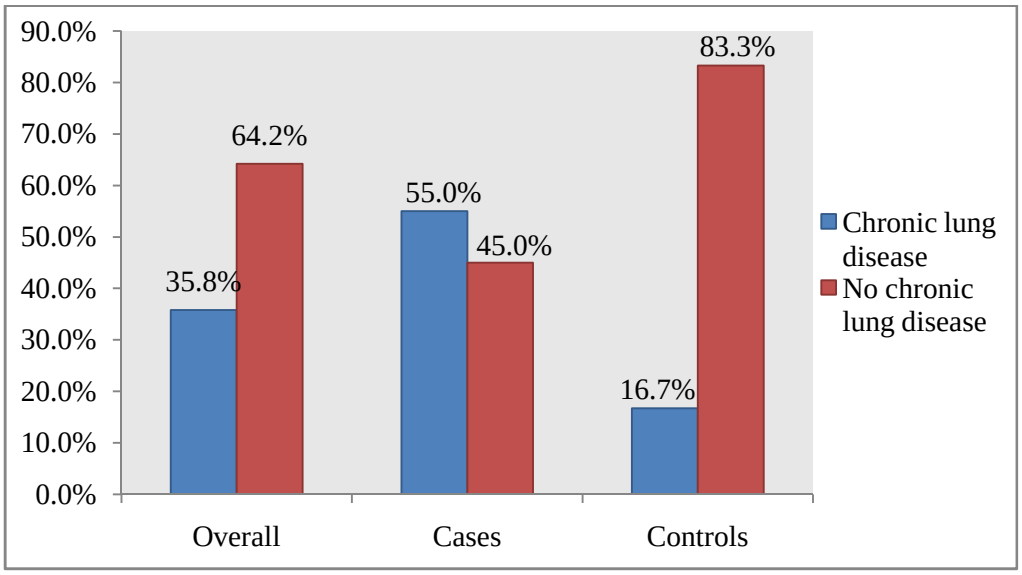


Figure 6: Presence of Chronic lung disease

8.6 The spectrum of chronic lung disease.

Pulmonary tuberculosis was the commonest diagnosed chronic lung disease in both cases, 18 out of 33, (54.5%) and controls 5 out of 10, (50%). The cases had more incidences of atypical pneumonia (5 patients) than the controls who were 3 patients. Bronchiectasis was found in 4 patients with finger clubbing who had previously been treated for PTB. Two cases and one control that were on continuation phase of PTB treatment were diagnosed with pneumocystis jirovecii pneumonia. Co-existence of PTB and LIP was also diagnosed in one patient with finger clubbing. [Table 3].

Table 3: Spectrum of chronic lung disease.

The spectrum of chronic lung disease	Cases n=33	Controls n=10	P value
Pulmonary tuberculosis	18 (54.5%)	5 (50.0%)	0.801
Atypical pneumonia	5 (15.2%)	3 (30.0%)	0.290
Bronchiectasis	3 (9.1%)	0 (0.0%)	0.323
Bronchiectasis with cor pulmonale	1 (3.0%)	0 (0.0%)	0.578
Persistent pneumonia	0 (0.0%)	1 (10.0%)	0.066
Persistent pneumonia with cor pulmonale	3 (9.1%)	0 (0.0%)	0.323
Pneumocystis jirovencii pneumonia with underlying PTB	2 (6.1%)	1 (10.0%)	0.668
PTB with lymphoid interstitial pneumonitis co-infection	1 (3.0%)	0 (0.0%)	0.578

8.7 The complications of chronic lung disease.

Of all the patients studied, 43 (35.8%) and 38 (31.7%) had abnormal oxygen saturations and pulmonary pressure respectively. The cases had 2.6 times risk of being diagnosed with mild to moderate hypoxemia, 28 (46.7%), OR 2.6 (95% CI 1.2-5.7), p=0.013, and 4.4 times risk of mild to severe pulmonary hypertension, 28 (46.7%), OR 4.4 (95% CI 1.9-10.2), p=0.001 as compared to the controls [Table 4]. An example of severe pulmonary hypertension on echocardiography is demonstrated in figure 7 below.

Table 4: Complications of chronic lung disease

Variable	Overall n=120	Case n=60	Control n=60	OR (95% CI)	P value
Oxygen saturations					
80-92% Mild/Moderate	43 (35.8)	28 (46.7)	15 (25.0)	2.6 (1.2-5.7)	0.013
93-100% Normal	77 (64.2)	32 (53.3)	45 (75.0)	1.0	
Pulmonary pressure					
Normal	82 (68.3)	32 (53.3)	50 (83.3)	1.0	
Mild/Moderate/Severe	38 (31.7)	28 (46.7)	10 (16.7)	4.4 (1.9-10.2)	0.001

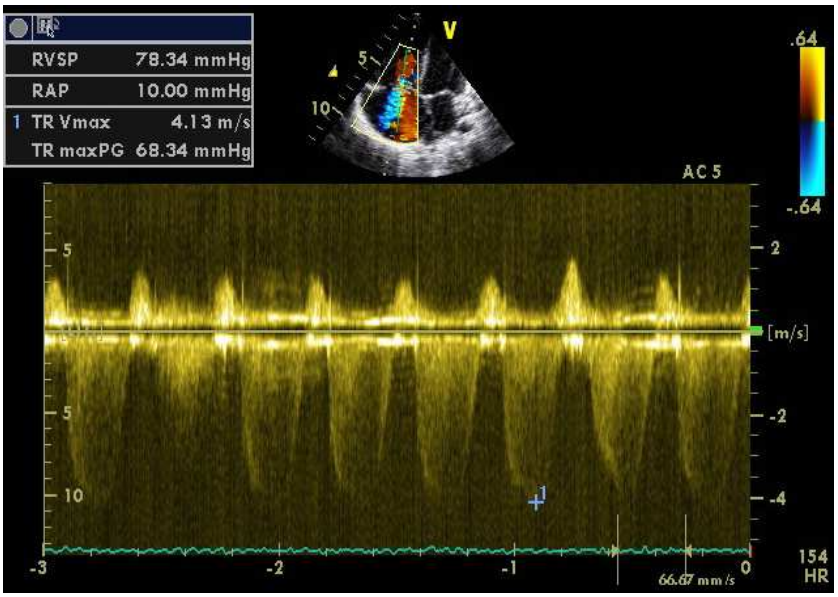


Figure 7: Sample Echocardiogram of severe pulmonary hypertension.

8.8: HIV WHO clinical staging at enrollment to the study.

Overall, 96 (80.7%) of the patients had WHO clinical stage III and IV. The number of patients with WHO stage III and IV disease was 6.4 times higher among the cases than the controls, OR 6.4 [95% CI 2.0-20.2], p<0.001 [Figure 8].

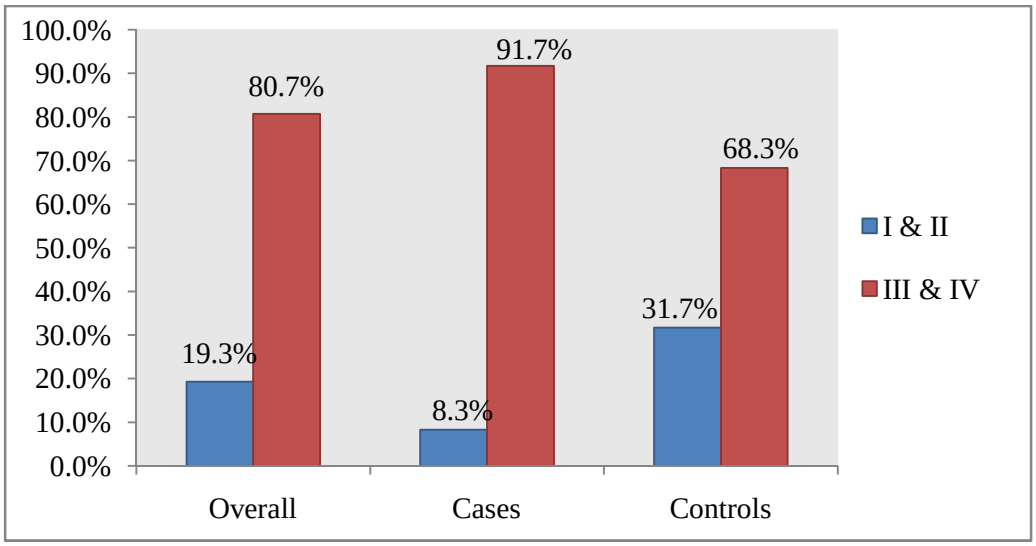


Figure 8: WHO clinical staging.

8.9. CD4 cell count/ percentage and antiretroviral use.

The cases had a significantly lower CD4 cells count (median 369 cells) compared to the controls (median 861 cells), p<0.001. Similarly, the CD4% for the cases was lower (median 13%) than that of the controls (median 28%), p<0.001. [Table 5]

Majority, 94 (78.3%) of the patients were using antiretroviral therapy and there was a trend towards higher percentage of antiretroviral use among the controls, 51(85%) compared to the cases 43(71.7%), p=0.076. However, duration of antiretroviral therapy was significantly different between cases and controls (p<0.001). The cases had a shorter duration of antiretroviral use (median 5.5 months) compared to the controls (median 40 months) [Table 5].

Table 5: CD4 cells count and Antiretroviral therapy treatment.

Variable	**Overall n=120**	**Case n=60**	**Control n=60**	**OR (95% CI)**	**P value**
Median CD4 count (IQR)	567(290-1057)	369 (103-708)	861(505-1165)	-	<0.001
Median CD4 % (IQR)	21 (11-31)	13 (5-23)	28 (19-35)	-	<0.001
Antiretroviral therapy					
Yes	94 (78.3)	43 (71.7)	51 (85.0)	0.4 (0.2-1.1)	0.076
No	26 (21.7)	17 (28.3)	9 (15.0)	1.0	
Antiretroviral therapy duration	24 (1-48)	5.5 (0.0-33.5)	40 (10.5-60)	-	<0.001

8.10 Incidental echocardiography findings among the participants.

Abnormal echocardiography findings other than pulmonary hypertension were found in 15 (12.5%) of study participants as incidental findings. Thirteen of these patients had finger clubbing. Pericardial effusion with or without constrictive pericarditis was demonstrated in nine patients, eight of whom had finger clubbing**.** Others findings included dilated cardiomyopathy and myocarditis as shown below [Table 6]. None of these patients was previously known to have an underlying cardiac pathology.

Table 6: Incidental Echocardiography Findings

Variable	**Cases n=x**	**Controls n=x**
Dilated cardiomyopathy	2	1
Dilated cardiomyopathy poorly contractile Right Ventricle	1	0
Pericardial effusion	5	1
Constrictive pericarditis	3	0
Myocarditis	2	0
Total	13	2

9.0 DISCUSSION

This study was carried out to determine association between finger clubbing and chronic lung disease in HIV infected children at Kenyatta National Hospital. The study was also set out to define how WHO clinical staging, CD4 counts and percentage and antiretroviral use correlate with finger clubbing.

This study has demonstrated that finger clubbing in children is associated with HIV infection and chronic lung disease accompanied by higher likelihood of pulmonary hypertension and hypoxemia. This is similar with other studies done in Malawi, South Africa and Zimbabwe (32-3, 39).

In this study, patients with finger clubbing were younger with a median age of 6.5 years compared to 9.0 years for those without finger clubbing. The youngest patient with finger clubbing in this study was nine months. Graham et al (31) too reported that finger clubbing in HIV infected children may occur as early as infancy.

This study reveals that, HIV infected children with finger clubbing are more likely to have chronic lung disease as compared to those without finger clubbing. This is similarly reported by Ferrand et al (39) from Harare where 10% of HIV infected adolescents with chronic lung disease had finger clubbing and in Nigeria, 15% of HIV infected adults with pulmonary complications had finger clubbing (40). However, these two studies were observational without comparison of the non finger clubbed group. This is because chronic lung disease is common in HIV infected patients and a cause of finger clubbing.

Tuberculosis was the commonest diagnosed chronic lung diseases in both finger clubbed and non finger clubbed patients in this study. This was similar to the Ugandan adult study by Ddungu et al (36). This may be due to the fact that Kenya is a high TB prevalence area and this trend is similar in other Sub-Saharan African countries where HIV and TB co-infection is high (39,41). However, in a South African study, the commonest chronic lung disease associated with finger clubbing was reported to be lymphocytic interstitial pneumonia (32). The reason for the difference could be due to the fact that in South Africa, they were able to do all appropriate investigations to differentiate between TB and LIP.

The other chronic HIV-associated lung disease associated with finger clubbing found in this study included lymphocytic interstitial pneumonia, persistent and atypical pneumonia,

bronchiectasis, and pneumocystis jirovecii pneumonia. The spectrum of chronic lung diseases in HIV in this study is similarly reflected by another study by Jeena et al (42).

In this study, 56% of all participants had been treated previously for a lung disease. This applied to both finger clubbed and non finger clubbed patients. This is due to the fact that in children with HIV infection, lung disease appears to be common as has been reported in other studies (22, 26, and 43).

It was also noted from this study that finger clubbed patients had six times likelihood of having abnormal chest radiological findings compared to non clubbed patients. Many other studies have reported similar findings. Norton et al (18) for instance reported the cumulative incidence of chronic radiographic lung changes in HIV-1–infected children to be 32.8% by 4 years old. Ferrand et al (39) also reported that 47% of long time survivors of vertically acquired HIV had subtle chest radiographic abnormalities. In another study by Desai et al (44), chest radiographic abnormalities were highly prevalent in adolescents with vertically-acquired HIV infection. This emphasizes that respiratory conditions are very common in HIV infected persons. However, these studies did not compare the chest abnormalities in regard to presence or absence of finger clubbing.

This study showed that approximately half (46.7%) of the HIV infected patients with finger clubbing had mild to moderate hypoxemia compared to a quarter (25%) of non clubbed patients. In the study by Graham et al (26), only 12.5% of the HIV infected children with finger clubbing had hypoxemia (oxygen saturation less than 90% on pulse oximetry). Our study had higher percentage compared to Graham et al (26) because our cut off for hypoxemia was oxygen saturations of less than 92%. Hypoxemia may be due to respiratory compromise in these patients. The study by Graham et al (26) however, did not also have a comparison group of non finger clubbed patients.

In this study, pulmonary hypertension was found to be more common in patients with finger clubbing. About 46.7% of finger clubbed patients had pulmonary hypertension compared to 16.7% of the patients without finger clubbing who had pulmonary hypertension. Two (7%) of patients with finger clubbing had severe pulmonary hypertension (>70mmHg). In both finger clubbed and non finger clubbed patients, mild pulmonary hypertension was predominant. There were no similar studies for comparison in regards to HIV infected children with finger clubbing and development of pulmonary hypertension. However, in

unpublished study by Kiptum et al (45), pulmonary hypertension with right ventricular dysfunction was found in 15% of children with AIDS at KNH. This is high compared to the Zimbabwe study by Ferrand et al (39), which found pulmonary hypertension in 7% of long-term survivors of vertically acquired HIV infection.

In this study, the occurrences of other incidental cardiac manifestations on echocardiography examination were 15 (12.5%). Thirteen of these patients had finger clubbing. This percentage is low compared to other studies that were looking for cardiac manifestations in HIV infection. For instance, by Kiptum et al (45) reported a prevalence of 65%, Okoromah et al (46) 75.9% and Miller et al (47) more than 50% with cardiac abnormalities in HIV/AIDS infected children. In our study, we were excluding known cardiac diseases like congenital heart lesions and rheumatic heart disease as they are known causes of finger clubbing.

It was found that finger clubbing was significantly associated with advanced disease, WHO clinical stage of III and IV. It is also worth noting that all the chronic lung disease in HIV fall in this WHO stages (48). However, this differed from an adult study by Ddungu et al (36) who reported that finger clubbing was not associated with the stage of HIV infection. There were no other similar studies in the paediatric population for comparison.

The finger clubbed patients were more likely to be hospitalized and had a lower CD4 counts and percentage as found in this study. This, therefore, means that patients with finger clubbing are more likely to be very sick requiring hospitalization compared to the ones without finger clubbing. Similar findings were reported by Zar et al (32) in South Africa that, HIV infected children with finger clubbing had lower CD4 count levels compared to those without finger clubbing.

In our study, the patients with finger clubbing had a shorter duration of antiretroviral therapy use (median 5.5 months) compared to the patients without finger clubbing (median 40 months). However, there were no other similar studies available for comparison.

10.0 CONCLUSIONS

1. Finger clubbing in HIV infected children is a pointer to the existence of chronic lung disease as a differential diagnosis.
2. The presence of finger clubbing in HIV infected children is associated with advanced WHO stage III or IV, lower CD4 counts and percentage and a shorter duration of antiretroviral therapy use, in other words, delayed initiation of antiretroviral therapy.
3. HIV infected children with finger clubbing have a higher likelihood of developing pulmonary hypertension.

11.0 RECOMMENDATIONS

1. In view of these findings, all HIV infected children must be examined for the presence of finger clubbing.
2. All HIV infected children with finger clubbing should have physical, radiological evaluation and echocardiography to asses for chronic lung disease and its complications.
3. All HIV infected children with finger clubbing should be planned for initiation of antiretroviral therapy.

12.0 STUDY LIMITATIONS

1. Lack of specialized additional investigations like high resolution CT scan of the chest and culture of bronchial aspirates may have assisted with more definitive diagnosis.
2. Post mortem was not done on those who died. This could have additionally revealed all the lung pathology not discovered in life, and further laboratory work up would have demonstrated presence or absence of chronic lung pathology in HIV infected children with finger clubbing.

13.0 REFERENCES

1. Tosti A, Piraccini BM. Nail disorders. In: Bolognia JL, Jorizzo JL, Rapini RP, editors. Dermatology. London: Mosby; 2003. p.1069.
2. Myers KA, Farquhar DR. Does this patient have clubbing? JAMA 2001; 286: 341–7.
3. Hansen-Flaschen J, Nordberg J. Clubbing and hypertrophic osteoarthropathy. Clin Chest Med 1987; 8:287–98.
4. Graham SM. Impact of HIV on Childhood Respiratory Illness: Differences between Developing and Developed Countries. Pediatric Pulmonology 2003; 36:462–8.
5. Kouakoussui A, Fassinou P, Anaky MF, Elenga N, Laguide R, Wemin ML, Toure R, Menan H, Rouet F, Msellati P. Respiratory manifestations in HIV-infected children pre- and post-HAART in Abidjan, the Ivory Coast. Paediatr Respir Rev 2004; 5:311-5.
6. Madhivanan P, Mothi SN, Kumarasamy N, Yepthomi T, Venkatesan C, Lambert JS, Solomon S. Clinical manifestations of HIV infected children. Indian J Pediatr 2003; 70:615-20.
7. Perez MS, Van Dyke RB. Pulmonary infections in children with HIV infection. Semin Respir Infect 2002; 17: 33-46.
8. Vandemergel X, Renneboog B. Prevalence, aetiologies and significance of clubbing in a department of general internal medicine. Eur J Int Med 2008; 19: 325–9.
9. Dickinson CJ, Martin JF. Megakaryocytes and platelet clumps as the cause of finger clubbing. Lancet. 1987; 2: 1434-5.
10. Dickinson CJ. The aetiology of clubbing and hypertrophic osteoarthropathy**.** Eur J Invest 1993;23: 330-8
11. Spicknall K E, Zirwas M, English III J C**.** Journal of the American Academy of Dermatology, 2005; 52:1020-8.
12. Martinez-Lavin M. Exploring the Cause of the Most Ancient Clinical Sign of Medicine: Finger Clubbing. Seminarsin Arthritis and Rheumatism 2007; 36:380-5.
13. Schwartz RA, Richards MG, Goyal S. Clubbing of the Nails. Medscape reference updated on April 25, 2011.
14. Altman RD, Tenenbaum J. Hypertrophic osteoarthropathy. In: S. Ruddy, E.P.J. Harris and C.B. Sledge, Editors, Kelly's textbook of rheumatology (6th ed.), WB Saunders, Philadelphia (2001), p. 1589.

15. Finger / Digital Clubbing http://www.surgicalnotes.co.uk/conte...gital-clubbing.
16. . Wilson GM. Local circulatory changes associated with clubbing of the fingers and toes. Q J Med 1952; 21 :201–13.
17. Mendlowitz M. Clubbing and hypertrophic osteoarthropathy, Medicine 1942; 21: 269–306.
18. Paton JY, Bautista DB, Stabile MW, Waldman AE, Nassar AG, Platzker AC, Keens TG. Digital clubbing and pulmonary function abnormalities in children with lung disease. Pediatr Pulmonol 1991; 10:25–9.
19. Pitts-Tucker TJ, Miller MG, Littlewood JM. Finger clubbing in cystic fibrosis. Arch Dis Child 1986; 61:576–9.
20. Pitrez PM, Jones MH, and Stein RT. HIV and the Lung in Developing World. Pediatric Pulmonology, Supplement 2004; 26:62–3.
21. Norton KI, Kattan M, Rao JS, Cleveland R, Trautwein L, Mellins RB, Berdon W, Boechat MI, Wood B, Meziane M, ACG . Chronic radiographic lung changes in children with vertically transmitted HIV-1 infection. Am J Roentgenol. 2001; 176:1553-8.
22. Berdon WE, Mellins RB, Abramson SJ, Ruzal-Shapiro C. Pediatric HIV infection in its second decade--the changing pattern of lung involvement. Clinical, plain film, and computed tomographic findings. Radiol Clin North Am. 1993; 31:453-63.
23. Akolo C, Ukoli CO, Ladep GN, Idoko JA. The clinical features of HIV/AIDS at presentation at the Jos University Teaching Hospital. Niger Med 2008; 17: 83-7.
24. Merchant RH, Oswal JS, Bhagwat RV, Karkare J. Clinical profile of HIV infection. Indian J Pediatr 2001; 38:239-46
25. Zar JH. Chronic Lung Disease in Human Immunodeficiency Virus (HIV) infected children. Paediatric Pulmonology 2008; 43:1-10.
26. Mouzinho A. Pulmonary Complications of HIV. Pediatric Pulmonology, Supplement 2004; 26:57–8.
27. Swaminathan S. Tuberculosis in HIV-infected children. Paediatr Respir Rev 2004; 5:225-30.
28. Berman DM, Mafut D, Djokic B, Scott G, Mitchell C. Risk factors for the development of bronchiectasis in HIV-infected children. Pediatric Pulmonology 2007; 42:871-5.

29. Chang AB, Redding GJ and Everard ML. Chronic Wet Cough: Protracted Bronchitis, Chronic Suppurative Lung Disease and Bronchiectasis. Pediatric Pulmonology 2008; 43:519–31.
30. Munro KA, Peter W. Reed PW, Joyce H, Perry D, Twiss J, Byrnes CA and Edwards EA. Do New Zealand Children With Non-Cystic Fibrosis Bronchiectasis Show Disease Progression? Pediatric Pulmonology 2011 46:131–8.
31. Graham SM, Daley HM, Ngwira B. Finger clubbing and HIV infection in Malawian children. Lancet 1997; 349**:** 31.
32. Zar HJ, Hussey G. Finger clubbing in children with human immunodeficiency virus infection. Ann Trop Paediatr. 2001; 21:15-9.
33. Dever LL, Matta JS. Digital clubbing in HIV-infected patients: an observational study. AIDS Patient Care STDS. 2009; 23:19-22.
34. Smyth A, Roberts N, Parker S, Tong CYW, Hart CA. Finger clubbing as a sign of HIV infection. The Lancet, 1997; 349:575.
35. Cribier B, Mena ML, Rey D, Partisani M, Fabien V, Lang J, Grosshans E. Nail Changes in Patients Infected With Human Immunodeficiency Virus. Arch Dermatol. 1998; 134:1216-20.
36. Ddungu H, Johnson JL, Smieja M, Harriet Mayanja-Kizza H. Digital clubbing in tuberculosis – relationship to HIV infection, extent of disease and hypoalbuminemia. BMC Infectious Diseases 2006; 6:1471-2334.
37. Jubran A. Pulse oximetry. Crit Care. 1999; 3: 11-17.
38. Ministry of Public Health and Sanitation. Kenya National Paediatric Tuberculosis Care Guidelines; 2011.
39. Ferrand RA, Desai SR, Hopkins C, Elston CM, Copley SJ, Nathoo K, Ndhlovu CE, Munyati S, Barker RD, Miller RF, Bandason T, Wells AU,Corbett EL. Chronic Lung Disease in Adolescents With Delayed Diagnosis of Vertically Acquired HIV Infection. Clin Infect Dis 2012; 55:145–52.
40. Peters EJ, Essien OE, Immananagha KK, Inah GA, Philip-Ephraim EE, Agbulu RE. CD4 count levels and pattern of respiratory complications in HIV seropositive patients in Calabar Nigeria. Niger J Physiol Sci 2007; 22: 93-7.

41. Bhat GJ, Diwan VK, Chintu C, et al. HIV, BCG and TB in children: a case control study in Lusaka, Zambia. J Trop Pediatr. 1993; 39:219-23.
42. Jeena PM, Coovadia HM, Thula SA, Blythe D, Buckels NJ, Chetty. R Persistent and chronic lung disease in HIV-1 infected and uninfected African children. AIDS. 1998; 12:1185-93.
43. Graham SM. Non-tuberculosis opportunistic infections and other lung diseases in HIV-infected infants and children. Int J Tuberc Lung Dis 2005; 9:592–602.
44. Desai SR, Copley SJ, Barker RD, Elston CM, Miller RF, Wells AU, Munyati S, Nathoo K, Corbett EL, Ferrand RA. Chest radiography patterns in 75 adolescents with vertically-acquired human immunodeficiency virus (HIV) infection. Clin Radiol 2011; 66:257-63.
45. Kiptum LD. Cardiac Manifestations in Children with acquired immunodeficiency syndrome at Kenyatta National Hospital. Mmed Dissertation, University of Nairobi, 2004.
46. Okoromah CA, Ojo OO, Ogunkunle OO.Cardiovascular dysfunction in HIV-infected children in a sub-Saharan African country: comparative cross-sectional observational study. J Trop Pediatr 2012; 58: 3-11.
47. Miller RF, Kaski JP, Hakim J, Matenga J, Nathoo K, Munyati S, Desai SR, Corbett EL, Ferrand RA.Cardiac disease in adolescents with delayed diagnosis of vertically acquired HIV infection. Clin Infect Dis 2013; 56: 576-82.
48. World Health Organization. WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. WHO, 2007.

14. APPENDICES

Appendix 1: PATIENT INFORMATION

Appendix 1.1 INVESTIGATOR'S STATEMENT

Dear participant,

My name is Dr .Justine Odionyi, a master's student at the University of Nairobi, school of medicine. I am carrying out research on the association and clinical correlates of chronic lung disease and finger clubbing in HIV infected children at Kenyatta National Hospital. The results of this study will be used by health institutions to improve their service delivery and improve the management of HIV/AIDS patients in the long run. Study data will be coded so that it will not be linked to your name. All information obtained in the course of this study will be held in confidence. There are no direct benefits to you as an individual participant. I request to include you and your child as a participant. This is voluntary and your decision on whether to participate or not will not prejudice your child's care in any way. There are no risks involved except the time you will be answering questions, examination of the child and doing chest radiograph and echocardiogram investigations. The investigations done shall be at no cost to you as the participant.

For any questions/clarifications, contact the principle investigator on:

P.O BOX 102299-00101, Nairobi, Kenya

Telephone number: 072297905

Email address: **justinejelagat@yahoo.co.uk**

You can also contact KNH/UON-ERC on:

P.O BOX 19676, Nairobi, Kenya. Email address**: uonknh_erc@uonbi.ac.ke**

Appendix 1.2 INFORMED CONSENT FORM

PARTICIPANTS STATEMENT

I Mr./ Mrs/ Miss……..being a person aged 18 years and over, having read/ been explained to the above and in the knowledge that is voluntary do hereby give consent for myself/ my child to participate in this study.

I understand that I/ my child have the right to withdraw from the research at any time, for any reason, without penalty or harm.

……..

Patient/Parent/Guardian

Date……

…….

Child's signature if above 7years (Assent)

Date…….

……

Investigator's signature

Date……

Appendix 1.3 TAARIFA KWA MSHIRIKA

MAELEZO YA MPELELEZI

Kwa mshiriki;

Jina langu ni Dk. Justine Odionyi, mwanafunzi katika Chuo Kikuu cha Nairobi, shule ya dawa. Mimi nafanya utafiti kuhusu uhusiano ya ugonjwa sugu wa mapafu na finger clubbing kwa watoto walioambukizwa virusi vya ukimwi katika Hospitali ya Taifa ya Kenyatta. Matokeo ya utafiti huu itatumiwa na taasisi za afya kuboresha utoaji wa huduma yao na kuboresha usimamizi wa wagonjwa wa ukimwi. Habari kutoka hapa itabaki siri na kutumika tu kwa madhumuni ya utafiti. Hakuna faida ya moja kwa moja kwa wewe mshiriki binafsi. Ningependa kushirikisha wewe, mtoto wako katika utafiti huu. Uuamuzi wa kushiriki ni kwa hiari yako na kama hautashiriki, haitazuiya huduma kwa wewe/ mtoto wako kwa njia yoyote. Hakuna hatari ya kushiriki ila wakati wewe utachukua katika kujibu maswali na kufanya uchunguzi ya X-Ray ya kifua na echocardiogram ya moyo. Uchunguzi itakayofanyika itakuwa bila gharama yoyote kwako kama mshiriki.

Kwa maswali yoyote/ ufafanuzi zaidi, wasiliana na mpelezi kwa anwani ifatayo:

P.O BOX 102299-00101, Nairobi , Kenya.

Nambari ya simu: 0722697905

Anwani ya barua pepe: justinejelagat@yahoo.co.uk

Unaweza pia kuwasiliana na KNH/ UON-ERC kwa:

P.O BOX 19676, Nairobi, Kenya.

Anwani ya barua pepe: uonknh_erc@uonbi.ac.ke

Appendix 1.4 FOMU YA IDHINI

Taarifa ya mshiriki

Mimi Bwana/ Bi.....mwenye umri wa miaka 18 na zaidi, baada ya kusoma/ kuelezwa hapo juu na kufahamu, kwahiari yangu, natoa idhini kwa mwenyewe/ mtoto wangu kushiriki katika utafiti huu.

Ninaelewa kwamba mimi/ mtoto wangu nina haki ya kujiondoa katika utafiti wakati wowote, kwa sababu yoyote bila ya adhabu au madhara.

Sahihi ya mzazi au mlinzi wa mtoto... ... ... Tarehe ... ...

Sahihi ya mtoto zaidi ya miaka 7.....Tarehe.....

Shahidi: Jina ... ... wajibu ... . saini ... ...Tarehe.....

Appendix 2: QUESTIONNAIRE

Study Title: THE ASSOCIATION AND CLINICAL CORRELATES BETWEEN FINGER CLUBBING AND CHRONIC LUNG DISEASE IN HIV INFECTED CHILDREN.

Demographic data

Patient study no……Initial……Hospital no……

Case /control [ ] Case= 1, Control = 2

Date…… Site [ ] CCC= 1, Ward= 2

Date of birth……Sex [ ] Male =1, Female =2

Residence: Estate……City/Town……..

Occupation: Father……..

Mother……

Guardian…….

Age at diagnosis of HIV…… Date of diagnosis……

Medical History(present illness)

(Duration in weeks or months)

1. Presenting complain……

ii……..

iii…….

iv…….

2. Cough?

- Yes……duration…….
- Sputum production: Yes……Colour……
- No cough
- No sputum production……..

3 Difficulty in breathing

- Yes……Duration……..
- No…….

4 Chest pain

- Yes……..Duration…….
- No……..

5. Fever Yes…….Duration…….

 No……

6. History of contact with TB case

 Yes……..How long ago…….

 No……..

7.History of failure to thrive

 Yes

 No

8. Diagnosis of current illness……

ii……..

iii……..

9.Treatment modality/ drugs of current illness:

i……

ii……..

iii……

Medical History (past)

10. Previous treatment for lung disease

 Yes……..1. Date……..Diagnosis…….

2. Date…….Diagnosis……..

3. Date ……..Diagnosis……

 No……..

11. Previous Chest X Ray report if available…….

……

12. Other previous therapy/ Hospital admissions

 Yes……1. Date …….Diagnosis……

2. Date……Diagnosis…….

3. Date……Diagnosis…….

 No……..

13. CD4 count ……...CD4 % …….

14. WHO staging (Current) …….

15.ART therapy

- Yes……..Duration……Regimen……
- NO

Examination

16. Weight (kg)…….Height (cm)…….. Standard Deviation……..

17. SPO_2 at room air……

18. Pallor

- Yes
- No

19.Lymphadenopathy

- Yes…….Site…….
- NO

20 Parotid enlargement

- Yes
- NO

21. Finger clubbing:

I. Schamroth's sign (Tick the appropriate finding).

- Present
- Not present

Ii, Phalangeal depth index.

Right hand index finger

DPD	IPD	DPD/IPD

Left hand index finger

DPD	IPD	DPD/IPD

22. Grade of finger clubbing if present…….

23. Examination of Respiratory System

- Respiration rate
  - Tachypnea
  - Normal
- Cyanosis
  - Present
  - Absent
- Dyspnea
  - Present
  - Absent
- Chest wall abnormality
  - Present (specify)……
  - Absent
- Chest wall movements:
  - Symmetrical
  - Asymmetrical
- Lower chest wall indrawing
  - Present
  - Absent
- Tracheal position (central or deviated to the right/ left)……
- Chest expansion
  - Symmetrical
  - Asymmetrical
- Percussion: Resonance…….Area/Side…….

  Dullness……Area/Side……

  Pain and tenderness……..site …….
- Auscultation findings
  - Vesicular breath sounds (normal)……..
  - Crepitations……site……..
  - Rhonchi……site……

- Bronchial breathing……..site……

24. Other systemic exam…….….

- Spenomegally
  - Present
  - Absent
- Hepatomegally
  - Present
  - Absent

25. Sputum/ Gastric aspirate results if available ….

- Microscopy……
- Culture…….

26. Tuberculin skin test if done (in millimeter)…….

- Positive……mm
- Negative

Investigations

27. Hemogram results (within the last one month)

WBC……..

Neutrophil counts……

Lymphocyte counts……..

RBC……..

Hemoglobin……

MCV……

MCH…….

MCHC……..

Platelet counts…….

28. Erythrocyte sedimentation rate (if available)…….

29. Other investigations done

i……

ii…….

iii…….

30. Chest x-ray report

…….

……

31. Echocardiography report (Pulmonary pressures)……

……..

……

26. Doctors diagnosis of chronic lung disease/ investigators diagnosis

- Yes……

  ……..

- No

  …….

Appendix 3: WHO case definition for HIV infection in children (2007) (48)

a) Adults and children 18 months or older

HIV infection is diagnosed based on:

Positive HIV antibody testing (rapid or laboratory-based enzyme immunoassay). This is confirmed by a second HIV antibody test testing (rapid or laboratory-based enzyme immunoassay) relying on different antigen or on different operating procedures.

b) Children younger than 18 months:

HIV infection is diagnosed based on:

Positive virological test for HIV or its components (HIV-RNA or HIV-DNA) confirmed by a second virological test obtained from a separate determination taken more than four weeks after birth.

Positive HIV antibody testing is not recommended for definitive or confirmatory diagnosis of HIV infection in children until 18 months of age.

Appendix 4: WHO CLINICAL STAGING OF HIV/AIDS FOR CHILDREN WITH CONFIRMED HIV INFECTION (2007) (48)

CLINICAL STAGE 1

Asymptomatic

Persistent generalized lymphadenopathy

CLINICAL STAGE 2

Unexplained persistent hepatosplenomegaly

Papular pruritic eruptions

Fungal nail infections

Angular cheilitis

Lineal gingival erythema

Extensive wart virus infection

Extensive molluscum contagiosum

Recurrent oral ulcerations

Unexplained persistent parotid enlargement

Herpes zoster

Recurrent or chronic upper respiratory tract infections (otitis media, otorrhoea sinusitis or tonsillitis)

CLINICAL STAGE 3

Unexplained moderate malnutrition or wasting not adequately responding to standard therapy

Unexplained persistent diarrhoea (14 days or more)

Unexplained persistent fever (above 37.5□ C intermittent or constant, for longer than one month)

Persistent oral candidiasis (after first 6-8 weeks of life)

Oral hairy leukoplakia

Acute necrotizing ulcerative gingivitis or periodontitis

Lymph node tuberculosis

Pulmonary tuberculosis

Severe recurrent bacterial pneumonia

Symptomatic lymphoid interstitial pneumonitis

Chronic HIV-associated lung disease including bronchiectasis

Unexplained anaemia (<8g/dl, neutropenia (<0.5×109 per litre) and or thrombocytopenia (<50×109 per litre)

CLINICAL STAGE 4

Unexplained severe wasting, stunting or severe malnutrition not responding to standard therapy

Pneumocystis pneumonia

Recurrent severe bacterial infections such as empyema, pyomyositis, bone or joint infection or meningitis but excluding pneumonia)

Chronic herpes simplex infection (orolabial or cutaneous of more than one month's duration or visceral at any site)

Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs)

Extrapulmonary tuberculosis

Kaposi's sarcoma

Cytomegalovirus infection: retinitis or cytomegalovirus infection affecting another organ, with onset at age older than one month

Central nervous system toxoplasmosis (after one month of life)

Extrapulmonary cryptococcosis (including meningitis)

HIV encephalopathy

Disseminated endemic mycosis (coccidiomycosis or histoplasmosis)

Disseminated non-tuberculous mycobacterial infection

Chronic cryptosporidiosis (with diarrhoea)

Chronic isosporiasis

Cerebral or B-cell non-Hodgkin lymphoma

Progressive multifocal leukoencephalopathy

Symptomatic HIV-associated nephropathy or HIV-associated cardiomyopathy

Appendix 5: WHO CLINICAL STAGING FOR ADULTS AND ADOLESCENTS WITH CONFIRMED HIV INFECTION (2007) (48)

CLINICAL STAGE 1

Asymptomatic

Persistent generalized lymphadenopathy

CLINICAL STAGE 2

Unexplained moderate weight loss (<10% of presumed or measured body weight)

Recurrent respiratory tract infections (sinusitis, tonsillitis, otitis media and pharyngitis)

Herpes zoster

Angular cheilitis

Recurrent oral ulceration

Papular pruritic eruptions

Seborrhoeic dermatitis

Fungal nail infections

CLINICAL STAGE 3

Unexplained severe weight loss (>10% of presumed or measured body weight)

Unexplained chronic diarrhoea for longer than one month

Unexplained persistent fever (above 37. 5°C intermittent or constant, for longer than one month)

Persistent oral candidiasis

Oral hairy leukoplakia

Pulmonary tuberculosis (current)

Severe bacterial infections (such as pneumonia, empyema, pyomyositis, bone or joint infection, meningitis or bacteraemia)

Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis

Unexplained anaemia (<8 g/dl), neutropaenia (<0.5 × 109 per litre) and/or chronic thrombocytopaenia (<50 × 109 per litre)

CLINICAL STAGE 4

HIV wasting syndrome

Pneumocystis pneumonia

Recurrent severe bacterial pneumonia

Chronic herpes simplex infection (orolabial, genital or anorectal of more than one month's duration or visceral at any site)

Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs)

Extrapulmonary tuberculosis

Kaposi's sarcoma

Cytomegalovirus infection (retinitis or infection of other organs)

Central nervous system toxoplasmosis

HIV encephalopathy

Extrapulmonary cryptococcosis including meningitis

Disseminated non-tuberculous mycobacterial infection

Progressive multifocal leukoencephalopathy

Chronic cryptosporidiosis (with diarrhoea)

Chronic isosporiasis

Disseminated mycosis (extrapulmonary histoplasmosis or coccidiomycosis)

Recurrent septicaemia (including non-typhoidal *Salmonella*)

Lymphoma (cerebral or B-cell non-Hodgkin)

Invasive cervical carcinoma

Atypical disseminated leishmaniasis

Symptomatic HIV-associated nephropathy or symptomatic HIV-associated cardiomyopathy

Appendix 6: WHO immunological classification for established HIV infection (2007) (48)

HIV RELATED IMMUNODEFICIENCY	<11 months (CD4%)	12-35 Months (CD4%)	36-59 Months (CD4%)	≥5 years (absolute number per mm3 or CD4%)
None or not significant	>35	>30	>25	>500
Mild	30-35	25-30	>25	350-499
Advanced	25-29	20-24	15-19	200-349
Severe	<25	<20	<15	<200 or <15 %

Appendix 7: Standard operating procedure for CD4 count analysis using the FACS Count system

Principle

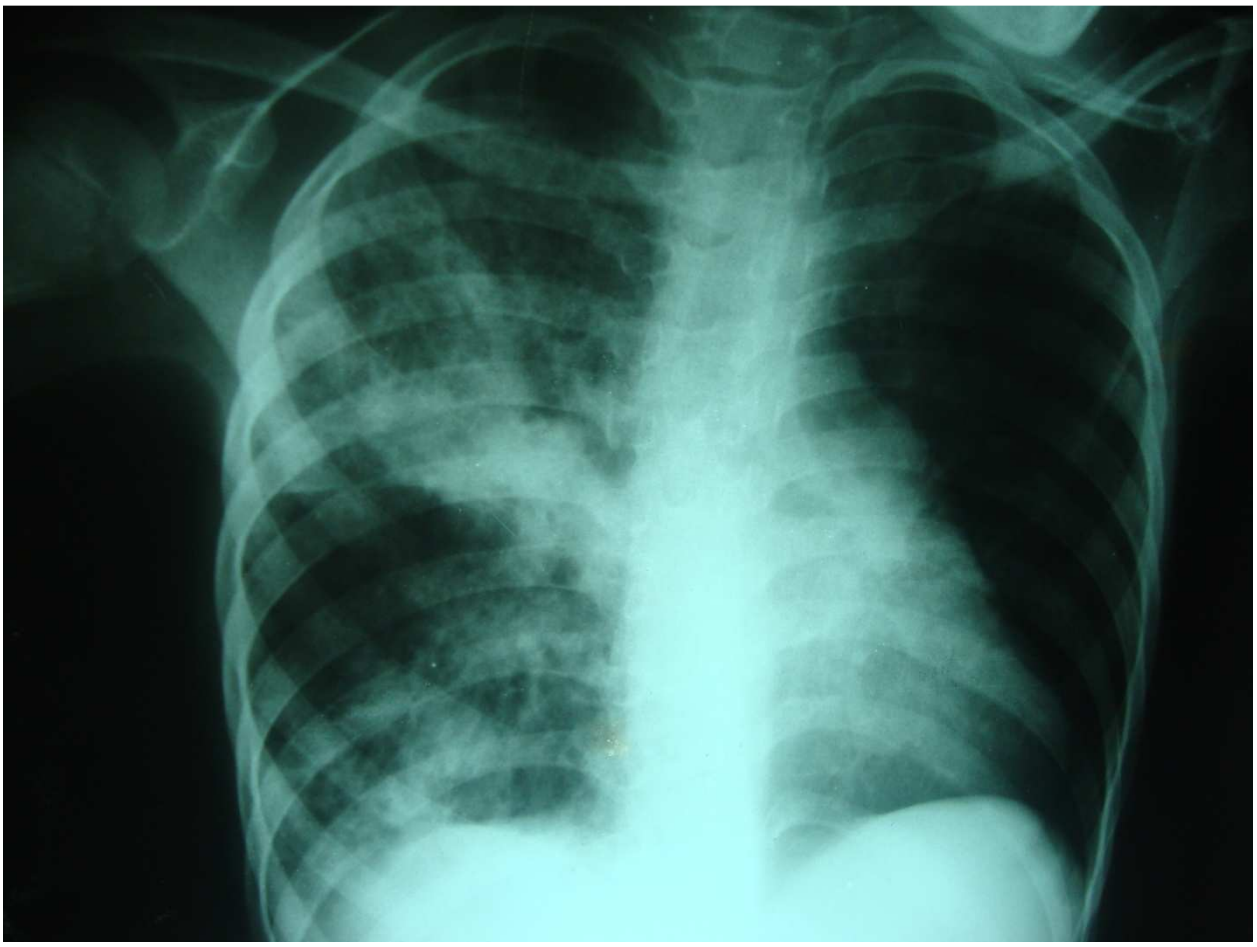
- A single test requires one convenient, ready- to- use reagent tube that determines the number of helper/inducer T-lymphocytes (CD4/CD3) and absolute number of total lymphocytes(CD3)
- When whole blood is added to the reagents, flourochrome-labelled antibodies in the reagents bind specifically to lymphocyte surface antigens.
- After a fixative solution is added to the reagent tubes the sample is run on the instrument. Here, the cells come into contact with the laser light, which causes flourochrome-labeled cells to fluoresce. This light provides the information necessary for the instrument to count the cells.

Procedure

- Patient blood samples are collected into EDTA vacutainer tubes and stored no longer than 48 hours at room temperature.
- Pipette 50ul of whole blood into the reagent tubes
- Cap tube and vortex upright for 5 seconds. Incubate the tubes for 60-120 minutes at room temperature.
- Uncap the tube and pipette 50ul of fixative solution into the reagent tube.
- Recap tube with new caps and vortex upright for 5 seconds
- Run the tubes on the FACSCount instrument.

Appendix 8: Sample Chest X-Ray pictures

CXR 1

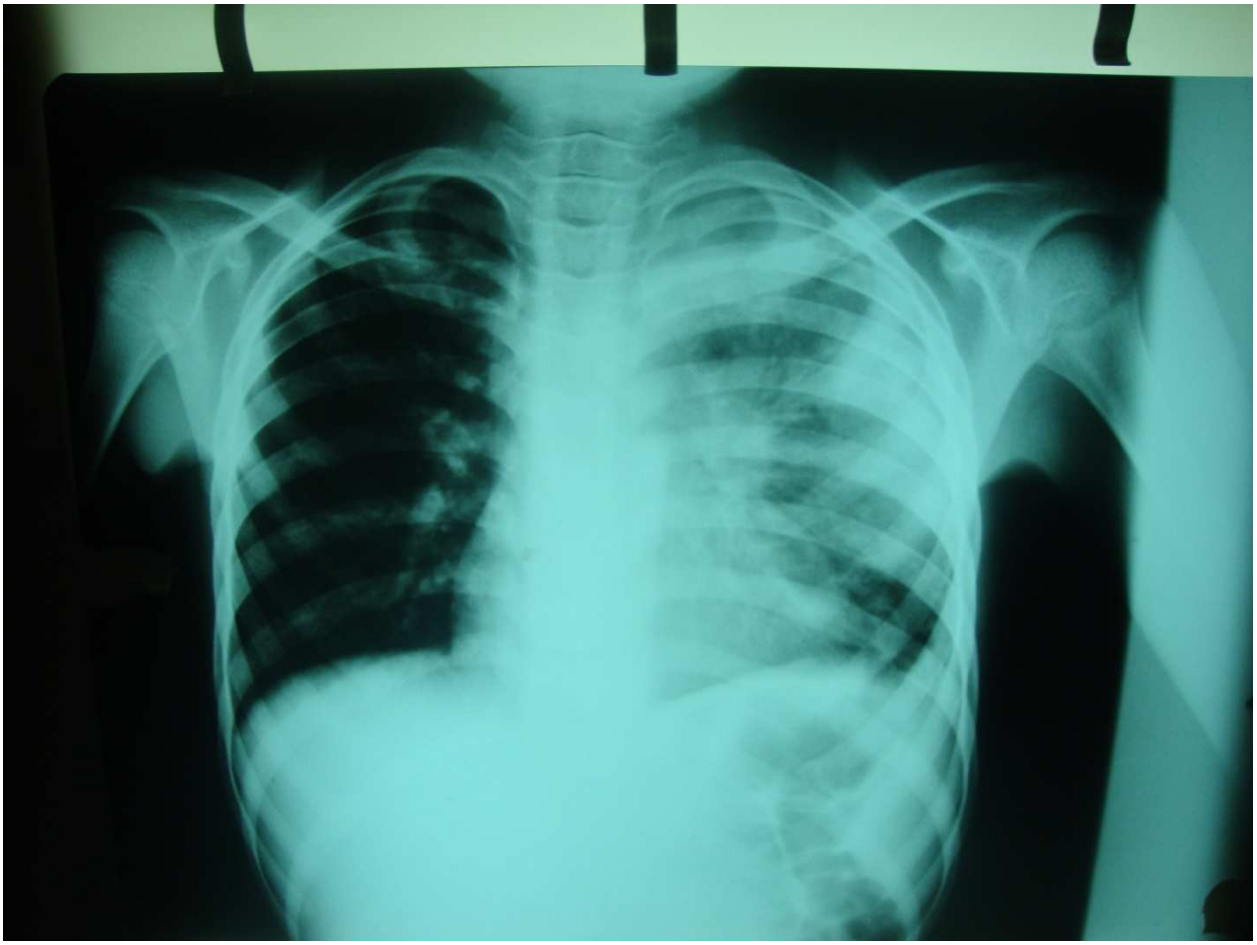


There are linear right upper lobe opacities with bronchiectatic changes on the right middle and lower lobes. The costophrenic angles are sharp. The cardiac size and thoracic bony cage are normal.

Conclusion: Bronchiectasis.

Echocardiography: moderate pulmonary hypertension ~51mmHg.

CXR 2



There is left lung opacification. There are patchy opacities in the right upper zone. Left hilar adenopathy is noted. The left costophrenic angle is blunted. The bony cage is normal and the cardiac size is normal.

Conclusion: Atypical pneumonia secondary to pulmonary tuberculosis.

Echocardiography: Mild pulmonary hypertension of 38mmHg.

Appendix 9: RESEARCH APPROVAL

UNIVERSITY OF NAIROBI
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KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
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Telegrams: MEDSUP, Nairobi
20th January 2012

Dr. Justine J. Odionyi
Dept. of Paediatrics & Child Health
School of Medicine
University of Nairobi

Dear Dr. Odionyi

Research proposal: "The association and clinical correlates of chronic lung disease in HIV infected children with finger clubbing at K.N.H." (P386/09/2011)

This is to inform you that the KNH/UON-Ethics & Research Committee has reviewed and approved your above revised research proposal. The approval periods are 20th January 2012 – 19th January 2013.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimens must also be obtained from KNH/UON-Ethics & Research Committee for each batch.

On behalf of the Committee, I wish you a fruitful research and look forward to receiving an executive summary of the research findings upon completion of the study.

This information will form part of the data base that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely

PROF A N GUANTAI
SECRETARY, KNH/UON-ERC

c.c. The Deputy Director CS, KNH
The Principal, College of Health Sciences, UON
The Dean, School of Medicine, UON
The Chairman, Dept.of Paediatrics & Child Health, UON
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"Protect to Discover"