ADHERENCE TO ARV AND ITS ASSOCIATION WITH IMMUNE STATUS AMONG HIV INFECTED CHILDREN AGED 2-14 YEARS IN DAR ES SALAAM.

By

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A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Medicine (Paediatric and Child Health) of the Muhimbili University of Health and Allied Sciences.

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CERTIFICATION

The undersigned certify that, they have read and hereby recommend for acceptance for a dissertation 'A***dherence to ARV and its association with immune status among HIV infected children aged 2-14 years in Dar-es-salaam'*** in partial fulfilment of the requirements for the degree of master of medicine (Paediatric and Child Health) of the Muhimbili University of Health and Allied Sciences.

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DECLARATION

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I, **Frida William Mghamba** declare that this **dissertation** is my own original work and that it has not been presented to any other university for similar or any other degree award.

Signature..... Date.....

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DEDICATION

I dedicate this work to my beloved husband Dr Joseph Hokororo for tireless support and encouragement during proposal and report writing. I also dedicate to my sons Chris and Billy, to my parents Mr William Mghamba and Mrs Juliana Mghamba.

ABSRACT

Background

Adherence is particularly critical with antiretroviral drugs in treatment of paediatric HIV infection where adherence of more than 95% is necessary to maximize the benefit of antiretroviral drugs. Studies on adherence in developed world have demonstrated that higher level of drugs adherence is associated with improved virological, immunological and clinical outcome. Despite the benefit of antiretroviral drugs in paediatric HIV infection, there are consequences to non adherence including disease progression, failure of viral suppression, decrease in CD4 cell count, drug resistance, risk of transmission of resistant virus and limited treatment options. It is therefore important to identify children with non adherence in order to intervene before developing drug resistance and treatment failure.

Objective

The aim of this study was to determine proportion of good adherence to ARV measured by caretaker report, medication return, plasma nevirapine concentration and its association with immune status among HIV-infected children in Dar es Salaam.

Methodology

A cross-sectional study was conducted between May and October 2011 in three Municipal hospitals (Mwananyamala, Temeke and Amana) in Dar es Salaam region. A total of 300 children aged 2-14 years on nevirapine based ARV regimen for at least six month were enrolled consecutively as they attend CTC. The study involved assessment of nutritional status and adherence to ARV. A single blood sample for CD4 count/percent and nevirapine plasma concentration was taken on the day of assessment. CD4 count was determined using flow cytometry and nevirapine plasma concentration was determined using reversed phase High Performance Liquid Chromatography (HPLC/UV). Proportions were used to summarise categorical variables and Chi square test as well as Fisher's exact test were used to test for statistical difference between these

variables. Mean and standard deviation was used to summarise continuous variables. A logistic regression model was used to assess the independent predictors of the outcome.

Results

A total of 300 children met inclusion criteria, the mean age (SD) of these children was 8(3) years and 50.7% were female. Caretaker report and medication return showed good adherence (98% and 97%) respectively. However adherence assessed by nevirapine plasma concentration was 85%significantly lower than that of caretaker report and medication return ($p<0.001$). Furthermore, the agreement between nevirapine plasma concentration and medication return and between nevirapine plasma concentration and self report were weak ($k=0.131$) ($k=0.09$) respectively. This means that care taker report and medication return reflected good adherence to ARV's which was contrary to what was found when using nevirapine plasma concentrations. Nevirapine plasma concentration below 3µg/ml was associated with immunosuppression ($p=0.021$) while medication return >5% of prescribed dose and caretaker reported missed dose were not associated with immunosuppression ($p=0.474$), ($p=0.569$) respectively. Therefore, nevirapine plasma concentration could be a predictor of adherence and correlate with immunosuppression when compared to medication return and caretaker report. Other independent factors associated with immune status were infections (OR=2.02, CI=1.01-3.41), Age of the child (OR=6.00, CI=2.96-12.17) and duration of ARV use (OR=6.79, CI=2.69-17.2).

Conclusions.

Nevirapine plasma concentration is a good predictor of adherence and correlate well with immunosuppression. Caretaker report and medication return were poor predictors of adherence. Non adherence by nevirapine plasma concentration was high (15%) suggesting that in every 10 children on ARV 2 did not adhere to medication. This is alarming considering the management of HIV and AIDS in paediatric patients.

Recommendations

Nevirapine plasma concentration should be used to assess adherence in those HIV infected children who develop clinical, immunological and virological failure. Qualitative studies are needed to explore the reasons for poor correlation between nevirapine plasma concentration and caretaker reported adherence as well as medication return and reasons for non adherence in paediatrics HIV care.

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LIST OF ACRONYMS

ABC	Abacavir
AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral Treatment
ARV	Antiretroviral
AZT	Zidovudine
CTC	Care and Treatment Clinic
ddI	Didanosine
d4T	Stavudine
EFV	Efavirenz
FDC	Fixed dose combination
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
ICOHTA	International Clinical Operation and Health services on TB and AIDS
LPV	Lopinavir
MDG	Millennium Development Goals
MDH	Management Development for Health
MTCT	Mother to Child Transmission

MUAC	Mid Upper Arm Circumference
MUHAS	Muhimbili University of Health and Allied Sciences
NACP	National AIDS Control Program
NVP	Nevirapine
OFC	Occipital Frontal Circumference
PCP	Pneumostic Jiroveci Pneumonia
PLWHIV	People Living With HIV
PTB	Pulmonary Tuberculosis
RTV	Ritonavir
SPSS	Statistical Package for Social Sciences
3TC	Lamivudine
WHO	World Health Organization

DEFINITION OF TERMS

ADHERENCE	A child is said to have good adherence if she/he missed no more than one dose, medication return ≤5% of prescribed dose and nevirapine plasma concentration > 3µg/ml.
CARETAKER	A person who lives with the child and participates in the child's daily care and take the responsibility in giving the child medication and bring the child to clinic.
CHILD	Person aged up to 14 years according to WHO.[1]
CONSECUTIVE SAMPLING	Non probability sampling technique which include all accessible subject as part of sample, considered as best of all non probability samples because it include all subject that are available.
HIV-INFECTED CHILDREN	Children aged more than 18 months with positive test for HIV antibodies using Enzyme immunoassays.
ANTIRETROVIRAL DRUGS	Drugs that inhibit replication of HIV.

1. 0 INTRODUCTION AND LITERATURE REVIEW

1.1 Epidemiology of HIV and AIDS

Human Immunodeficiency Virus (HIV) infection is one of the most destructive epidemics the world has ever witnessed. According to 2010 WHO global report on AIDS epidemic estimates the number of people living with HIV was 34 million, of these 22.9 million were from Sub Saharan African. Children continue to be born with HIV worldwide; however, sub-Saharan African is the most affected. Nightly percent of estimated 3.4 million children less than 15years living with AIDS were from sub Saharan African. Children less than 15 years newly infected with AIDS were 390,000. Although children under the age of 15 years represented about 14.8% of 22.9 million people living with HIV in Sub-Saharan African, they accounted for 13.8% of the 1.8 million death [1] and HIV/AIDS account for 9% of mortality in children aged below five years.[2]

Over I.4 million people are estimated to be living with HIV and AIDS in Tanzania, children below 14 years constitute 11.4%.[3] HIV and AIDS have impact to the health and economy of the country. Tanzania, being a resource limited country with an increase of death due to AIDS many children are left Orphans resulting into burden to the health system and economy of the country. In 2009, estimated AIDS deaths were 86,000 and orphans were 1million.[3] Among children below five years, mortality attributed to HIV was estimated to be 16 percent.[4] In 2002, prevalence of HIV among children admitted at Muhimbili National hospital, in Dar es Salaam was 19.2% and mortality rate was 21.4%.[5]

1.2 Tanzania HIV and AIDS care and treatment programs

Antiretroviral therapy introduced worldwide has resulted in a decrease in mortality and morbidity due to HIV and AIDS however the efficacy of antiretroviral therapy depend on high level of adherence. [6, 7] For infants and children Tanzania started providing antiretroviral therapy since early 2004. Care and treatment centres are responsible for

management of HIV/AIDS patients under the coordination of the National care and treatment program. Services provided at care and treatment centre (CTC) include providing antiretroviral therapy to eligible patients, counselling on adherence to ARV, treatment of opportunistic infection, nutrition counselling and growth as well as monitoring and evaluation. In 2007, Tanzania was one of the countries with the largest number of children on ARV (11%) in East and Central Africa.[8] Antiretroviral therapy suppresses viral load and raises the number of CD4 cell thus improving quality of life of HIV infected patients. ART is lifelong therapy and requires stringent adherence to treatment. Antiretroviral therapy with at least three drugs is recommended for treatment of HIV- infected children to preserve and improve immune function.[9]

Non nucleoside reverse transcriptase inhibitors are the WHO recommended backbone of first line antiretroviral therapy. In Tanzania most of children are on nevirapine based antiretroviral regimen. However data on factors influencing nevirapine pharmacokinetics and exposure are lacking. Nevirapine pharmacokinetic profile is characterized by long half life, 60% binding to plasma protein and elimination mainly through oxidative metabolism involving CYP 3A and CYP 2B6.[10] The role of CYP 3A on nevirapine metabolism is not clear and its frequency is low in African descent. The importance of CYP 2B6 genetic polymorphism in efavirenz metabolism is now well established but its effect influence on nevirapine metabolism is less clear.[11] A recent study done in Tanzania indicates low frequencies of hyplotype expressing increased activity of CYP 3A and CYP 2B6 among Tanzanian populations.[12]

1.3 Adherence to Antiretroviral therapy

Adherence is defined as ability of a patient to adopt behaviour and attitude that serve to empower him/ her to improve health and self manage a given illness or, ability of a patient to take all medications as prescribed with no missed dose; the right drugs, right dosage, right time, and right way.[13, 14, 15] Establishing and maintaining adherence to medication is a difficult goal for an individual with chronic illness even when treatment regime is simple and the patient is clearly symptomatic.[14] Even brief episode of missed

medication dose can permanently undermine HIV treatment leading to reduced efficacy and increase resistance to medication.[15, 16]

ARV treatment for children requires collaboration between the child and caregiver in terms of commitment of caregiver and cooperation of the child. Difficult taking ARV medication is due to unpleasant flavour, smell, nausea, too many pills and side effects .[17] Other factors associated with adherence are age, sex, caregiver type, income, disclosure to child, caregiver-child communication, caregiver health believe, depression, stress, stigma and forgetfulness.[18]

One major barrier to measuring and ultimately improve adherence is lack of any gold standard for measuring adherence. A number of measuring strategies exist including pill count, electronic monitors, diaries and report questionnaire. Each of these methods has limitations and can provide different estimate of adherence.[19,20] The limitations of these strategies have been more pronounced with children. For example regarding pill counts, many children have difficulties in swallowing pills and require liquid medication. Electronic monitors which record each time bottle has been opened are not currently practical with liquid formulation. Furthermore self report commonly used in most research studies and clinical work overestimates adherence to antiretroviral therapy due to recall bias.[21]

Adherence measurements in HIV patients in recent research have suggested that use of multiple measures will be of greater benefit than continue search for single defining adherence measure.[22] It is apparent that use of drug plasma concentration is the most accurate and objective way to assess adherence.[23] However the use of this method has logistic and cost implications. Consequences of non adherence are failure of viral suppression, decrease CD4 cell count, disease progression, drug resistance, risk of transmission of resistance virus and limited future treatment options. [24] Common reasons for non adherence are those related to patient, her/his family, related to drug and health system which include caretaker being too busy, forgetfulness, away from home, change in daily routine, fall asleep, illness, pill burden and side effect of ARV drugs.[25]

Strategies that may improve adherence to antiretroviral therapy include taking medication at the same time every day, associate with daily activity (meal, tooth brushing), simple treatment regimen, use of Fixed Drug Combination, use of alarm, watch pill boxes, reminder from treatment supporter and carrying extra dose when away from home.[26]

1.4 Proportion of good adherence to antiretroviral therapy

The data in paediatric adherence to ARV is limited studies on adherence to antiretroviral therapy in children and adolescent indicate that fewer than 50% of children and / or caretaker reported full adherence to their regimens.[27, 28]

In Kampala Uganda only 72% of children aged 2-18 years had adherence > 95% measured with home unannounced pill count compare to 89% using 3 day self reported adherence and 94% using clinic –based pill count.[29] While in a study done in South Africa on adherence to ARV in young infants and children using medication return 94% of children had good adherence.[30]Another study in South Africa on paediatric adherence using MEMS (medication event monitoring system) to monitor adherence show only 36% of patients achieved > 95% adherence in comparison to 91% of caregiver reporting excellent adherence on visual analogue.[31]

In Abidjan cote d' Ivoire assessment of adherence to HAART in cohort of African HIV infected children 33% reported less than full adherence . The commonest reasons for non -full adherence was forgetting to take treatment 40.5% [9]

Adherence to ARV therapy among HIV children in tertiary hospital in Nigeria was 86% according to caregiver report in three day preceding the interview and 14% of children did not adhere. The commonest reason for non adherence was drug stock- out 48.5% .[32]

Adherence measured by drug level is more objective and accurate but has been poorly documented worldwide. There is no data on adherence assessed by nevirapine plasma concentration among HIV infected children in Tanzania because it is expensive to be done routinely so it is done only in research settings.

Nevirapine trough plasma concentration of 3µg/ml has been shown to be a cut off for efficacy. This is the minimum therapeutic nevirapine plasma concentration below this cut point is associated with virological failure. The positive predictive value of these cut-off point was 55% and negative predictive value of 88%.[33] A recent adherence study done in Cameroon in adult HIV patients, indicated a higher level of adherence with respect to self report (97.5%) as compared to nevirapine plasma concentration (88%).[34]

1.5 Association between adherence and immune status

Adherence is a good predictor of effective virological suppression and subsequent immunological recovery. Sub-optimal adherence appears to decrease the likelihood of viral suppression and increase likelihood of drug resistance. Adherences more than 95% have been associated with virological suppression.[35] Studies done to establish association between adherence and immunological response among HIV-infected children have shown conflicting finding. Where a study done in Ethiopia on association of adherence to ARV and immunological response among HIV infected children show that caregiver self report on adherence was significantly associated with immunological response, [36] another study in South Africa showed that adherence in HIV infected children assessed by medication return was not associated with immune response.[30] In United States, adherence was assessed using self report (3days recall of dose missed) and pill count and there was no association between adherence and immune response.[37] Therefore, there is a need to determine the association between different adherence methods and immune status in our settings in order to provide evidence-based information for improving adherence to ARV particularly in the paediatric patient population.

1.6 Factors associated with immune status

Immune status in children is assessed by measuring CD4 cell count for children more than 5 years and CD4 percent for children below 5 years, children below 5 years have higher absolute CD4 count and vary with age reaching adult level at age of six years while CD4 percent remain relatively constant.[38] Low CD4 cell counts and percentage in children below 5 years are considered to be markers of progression of HIV- infection and AIDS. Low CD4 cell counts and percentage in children below 5 years increase likelihood of opportunistic infections.[39]

Malnutrition cause deterioration of immune status by affecting cell mediated immune response, compliment system and phagocytic function. When a malnourished child is challenged by infection, there is further weakening of immune response.[40] In India, severity of malnutrition was found to have an inverse correlation with CD4 cell count/ percentage.[41] In this study it was further observed that lower CD4 count/ percentage correlated with advanced WHO clinical stage.[41] Malnutrition cause depletion of CD4 count and this may exacerbate the progression of HIV infection.[42] Children with opportunistic infections have lower CD4 value compared to children without opportunistic infection as reported in one study conducted in India.[43]

Introduction of ARV for treatment of HIV infection have reduced mortality and morbidity in children. This is one of the strategies towards achieving MDG4 and MDG6 in high burden countries including Tanzania. However, to achieve the maximum benefits of ARV's good adherence to medication is very important.

Blood drug concentration is the most accurate and objective measure of adherence yet, adherence to antiretroviral therapy using blood drug a concentration has not been estimated in Tanzania. The main objective of this study was to determine proportion of good adherence to ARV measured by caretaker report, medication return, plasma nevirapine concentration and its association with immune status among HIV-infected children in Dar es Salaam.

2.0 PROBLEM STATEMENT

HIV/AIDS is the one of serious health problems and a leading cause of mortality and morbidity among children in Tanzania. Mortality rate due to HIV/AIDS in Tanzanian children below five years was reported to be 16% in 2002. At Muhimbili National Hospital prevalence of HIV among admissions was 19.2% and the mortality rate was 21.4%.[5] Introduction of antiretroviral therapy has resulted in decrease in mortality and morbidity in HIV infected children however, efficacy of antiretroviral therapy depends on high level of adherence.

There is limited data on adherence to antiretroviral therapy worldwide, few studies of HIV-infected children show adherence to antiretroviral drugs as a major problem in paediatric antiretroviral therapy. Adherence to antiretroviral drug in children and adolescents is a problem due to multiple factors which include high pill burden, poor palatability, side effects, long term toxicity, forgetfulness and caretaker factors.[17,18] Consequences of non adherence to antiretroviral drugs include increase in viral load, decrease of CD4 cell count, disease progression, ARV drugs resistance, risk of transmitting resistant viruses and limitation of future treatments options.[24] Therefore high level of adherence is very crucial to maximize the usefulness of antiretroviral therapy.

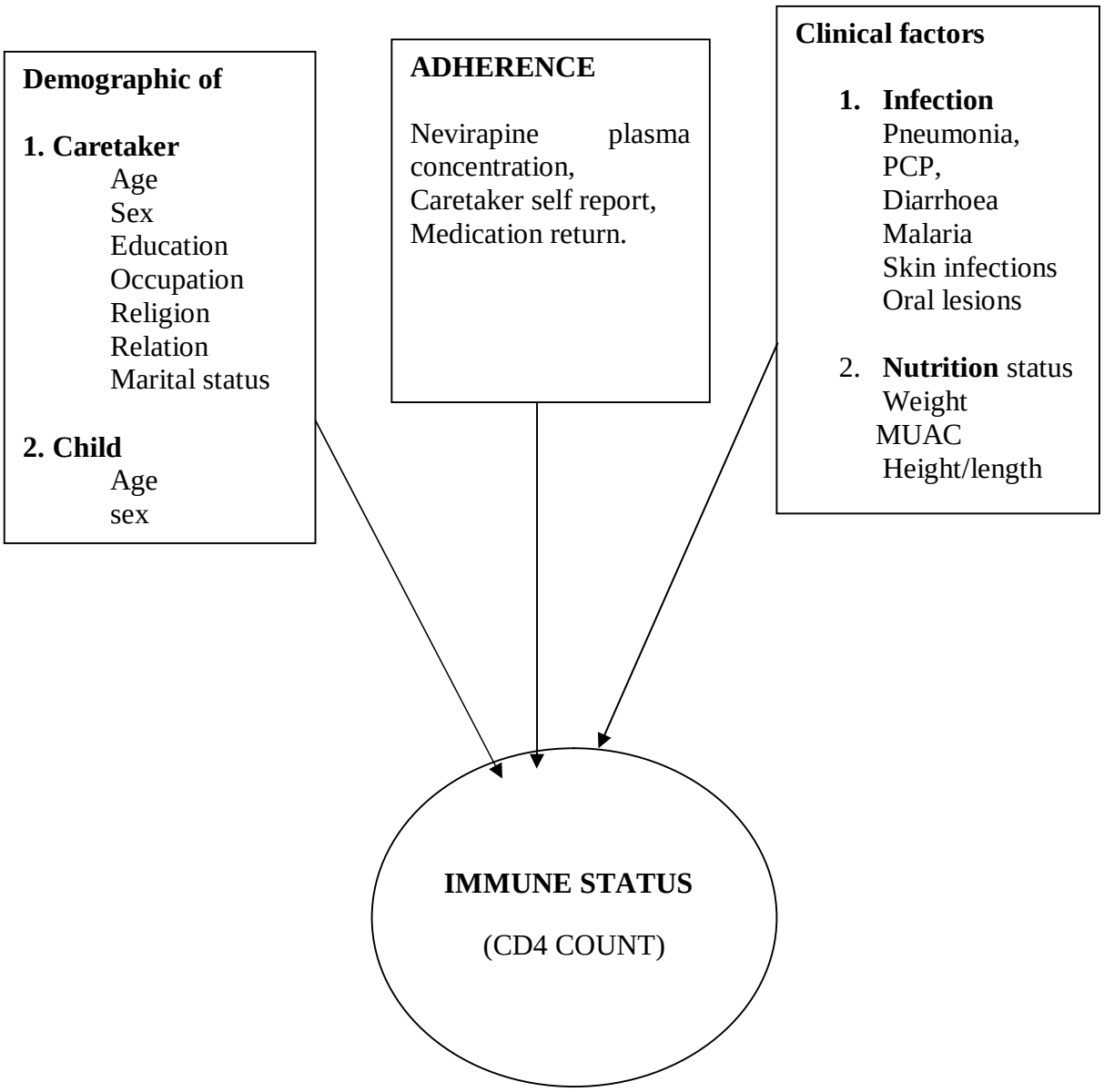
The level of adherence to ARV measured by blood drug levels and its association with immune status among HIV infected children in Tanzania has not been established. Most of studies in literature determined association between other measures of adherence (caretaker report and medication return) to antiretroviral therapy and immunological response. These methods have been reported to overestimate adherence particularly in HIV patients that are on medication for long time.[29, 34] To our knowledge, no study in Tanzania has been reported to apply blood drug levels measurements in assessing adherence to ART and its association with immune status in HIV infected children.

3.0 RATIONALE OF THE STUDY

With improved diagnostic tests, HIV status of many children can be confirmed early and starting ART early. ART is life-long, therefore it is important to assess level of adherence and look for accuracy of the methods currently used to assess adherence in children. Poor adherence will eventually reduce the efficacy of ARV and hence poor treatment outcome.

This study used an objective method to assess adherence to antiretroviral therapy (nevirapine plasma concentration) as a better estimation of proportion of good adherence to ARV. Findings of this study have given a good estimate of the current situation of adherence to ARV in paediatric patients in Tanzania. Understanding the level of adherence to ARV and immune status in children will contribute to improved adherence to ARV. Data from this study is useful to health planners such as those at the Ministry of Health and Social Welfare. This finding potentially enable Ministry of Health and Social Welfare design better programmes to alleviate the problem of non-adherence to ARV in children and serves as resource for new research on identified gaps.

4.0 CONCEPTUAL FRAMEWORK



The conceptual framework demonstrate the aim of this study to explore the proportion of good adherence as predicted by nevirapine plasma concentration, medication return and caretaker self report and its association with immune status as outcome. Other factors considered as possible confounders are demographic factors of caretaker, age and sex of child, clinical factors of child however, study will only focus on adherence and outcome of interest.

5.0 OBJECTIVES

5.1 Broad objective

The aim of the study was to determine the proportion of good adherence to ARV and its association with immune status among HIV infected children aged 2- 14 years in Dar es Salaam.

5.2 Specific objectives

1. To determine proportion of good adherence to ARV among HIV positive children in Dar es Salaam measured by medication return, caretaker report and nevirapine plasma concentration.
2. To compare adherence to antiretroviral drug measured by nevirapine plasma concentration versus caretaker report and medication return among HIV children in Dar es Salaam.
3. To establish association between adherence measures and immune status among HIV infected children in Dar-es-salaam after adjusting for age and sex of the child, duration of ARV, infections, disclosure status, WHO clinical stage and socio-demographic factors of caretakers.

5.3 Research questions

What is the proportion of good adherence to ARV among HIV-infected children aged 2-14years in Dar es Salaam?

Is there an association between adherence measures and immune status among HIV-infected children in Dar-es-Salaam?

5.4 Null hypothesis

There is no association between adherence to antiretroviral drugs and immune status among children in Dar es Salaam.

4.2 Alternative hypothesis

There is association between adherence to antiretroviral drugs and immune status among HIV children in Dar es Salaam.

6.0 METHODOLOGY

6.1 Study design

This study was a cross-sectional study among HIV children on antiretroviral drugs.

6.2 Study setting

This study was conducted in three paediatric HIV/AIDS Care and Treatment Clinics (CTCs) in Municipal hospitals in Dar es Salaam. Dar es Salaam region has three Municipal hospitals; Mwananyamala, Temeke and Amana. These three municipal hospitals are public hospitals with large number of HIV infected children attending Care and Treatment Clinic. Amana CTC has total of 5479 patients 468 are children below 15 years 80% of whom are on ARV. Mwananyamala CTC has total of 5577 patients 434 are children below 15 years 83% of whom are on ARV. Temeke CTC has total of 4989 patients 460 are children below 15 years 85% of whom are on ARV. Dar es Salaam has population of more than three million people 32% of whom are children under the age of 15 years.[44]

6.3 Study population

HIV infected children aged between 2-14 years on antiretroviral drugs for at least 6 months who attended CTC at Mwananyamala, Temeke and Amana from May 2011 to October 2011 and met inclusion criteria.

6.4 Selection criteria

6.4.1 Inclusion criteria

1. HIV infected children on nevirapine based regimen for at least six months

2. Aged between 2- 14 years who attended CTC at Mwananyamala, Temeke and Amana from May 2011 to October 2011 because at 2 years we expect the child would be diagnosed and to be on ARV for more than 6 months.

3. Children who caretakers provided informed consent to participate in the study.

6.4.2 Exclusion criteria

1. HIV infected children with severe malnutrition because malnutrition causes immunosuppression.

2. Children not on nevirapine based regimen.

6.5 Study duration

The duration of study was 5 months, from May 2011 to October 2011

6.6 Sample size estimation.

Since this study was descriptive, the formula for minimum sample estimation for descriptive studies was used.

$N=4P(100-P)/d^2$

Where N = minimum sample size

4= approximated squared value of standard normal distribution of 1.96 to 2 (at 95% CI)

P= estimated proportion of adherence to ARV (86% study done on HIV children in tertiary hospital Nigeria). [32]

d= maximum likely error (5%)

Hence the minimum sample size was calculated to be 193. In addition, sample size estimation for the analytical part of the study was calculated. Sample size calculation for **Analytical studies** where Adherence and immune status are dichotomous variable using two proportions was done using the formula for independent but unequal group size.

$$\mathbf{(Z1-\alpha/2 + Z1-\beta)^2 (\pi_0 (1-\pi)/no + \pi_1 (1-\pi_1)/n_1) = (\pi_1-\pi o)^2}$$

Proportion of children with good immune status (π_1) = 50% and proportion of children immunodeficiency π_0=30%

α = 0.05; power =0.80 β =1-0.80=0.20 ratio 1; 2

Minimum sample size was calculated to be 200 participants. In order to increase precision of study (results) a total of 300 participants were planned to be enrolled into the study.

6.7 Sampling procedure

A consecutive sampling procedure was used to recruit children into the study. All children who meet inclusion criteria and their caretaker provided written consent were recruited consecutively on their visiting day at the clinic on Friday. At the clinic recruitment was done by starting with provision of information regarding the study to the parent/caretaker after which they were requested to sign the consent form for participation in the study.The numbers of children on ARV in these sites almost the same; hence equal number (100) of children was recruited from each clinic.

6.8 Measurements

6.8.1 Predictor variable

Adherence and non adherence measured by caretaker report, medication return and nevirapine plasma concentration

6.8.2 Possible confounders

Possible factors that may affect adherence to ARV and immune status from literature review were : Demographic factors of caretaker (Age, sex, occupation, marital status, level of education, religion) ; age and sex of the child, caretaker type, disclosure to child, use of medication remainder, duration on ARV, adverse effects**.** Clinical factors: infections such as pneumonia, diarrhoea, malaria, otitis media, oral lesion, HIV stage, nutritional status.[17, 18, 40, 43]

6.8.3 Outcome variable

Immune status categorized as good immune status if CD4 % >30% for children below 5 years or CD4 count >500 for children above 5 years below that will be categorized as immunodeficiency according to WHO immunological classification.[45]

6.9.0 Data collection instruments

A structured questionnaire was developed in English and translated to Swahili. Training of research assistants was done at each site. Pilot testing of questionnaire was also done before the study began. Data collection tools were weighing scales, length boards, non stretchable tapes, WHO anthropometrics measurement chart, needles, and tubes for blood sample collection.

The following information was collected for eligible patients and whose parent/caretaker consented. Information on age and sex of the child, socio demographic of the caretaker, history of illness in previous visit and using other medication apart from ARV was asked and documented. History of cough, fever, ear discharge, vomiting medication, skin lesion and oral lesion was asked. Parent/ caretaker were asked for permission to do examination and to collect specimen. General and physical examination were done, weight, length and mid upper circumference measurement were taken to asses' nutritional status. Treatments were given to children according to diagnosis. The information on disclosure for children above 7years, duration on ARV were taken and type ARV regimen were checked to make sure they were on nevirapine regimen and correct dosage 160-200mg/m^2/dose twice daily according to Tanzania national guidelines for management of HIV and AIDS in children.[45]

Adherence was assessed by self report of parent or caretaker and children, medication return (pills or syrup) and nevirapine plasma concentration. Adherence to ARV was assessed using modified Swahili version of self reported adherence assessment tool developed by the Paediatrics AIDS Clinical Trial Group (PACTG).[46] Parent/caretaker

reported the number of missing doses on each day of 3 days prior to assessment. Good adherence when no more than one dose was missed in three days prior interview.

Adherence assessed by medication returned was categorized as good adherence when less than 5% of the medications were returned and non adherence when more than 5% of medications were returned. The level of adherence was calculated as percentage of prescribed dose not returned. Older children and caretaker of children identified to be non adherence to medication were educated and counsel on the benefit of adherence and consequences of non adherence and use of reminder for those who forgot to take medication.

Nevirapine plasma concentration was determined by HPLC method[47] and was used to assess adherence. The drug plasma concentration was compared with subjective measures of adherence self report and medication return. The amount blood sample was 4ml collected at cubital fossa 2ml for nevirapine plasma concentration and 2ml for CD4 count/percentage at (10.00- 12.00am) 4-6 hours after last dose. Blood for CD4 count/percentage were kept at EDTA tube transported to Muhimbili and analysed by flow cytometry. The bloods for nevirapine plasma concentration were kept in heparinised tubes and immediately centrifuged at 1300× g for 10 minutes. The obtained plasma was transferred into cryovials and transported in a cool box to MNH. The samples were stored at -70ºC until assay. Determination of nevirapine plasma concentration was done using a validated reversed phase High Performance Liquid Chromatography (HPLC) method with ultraviolet detection at the bio- analytical laboratory Unit of Pharmacology, school of Pharmacy at MUHAS. A reverse column C-18 was used and mobile phase consisted of 250ml acetonitrite and 800ml phosphate buffer (pH 7.5). Detection was achieved at wavelength 282nm and carbamazepine was used as internal standard.

6.9.1 Determination of Nevirapine plasma concentration

Before analysis of patient samples, the HPLC method was validated with respect to accuracy and precision. The method was also tested for lack of interference of endogenous substances and the components of cotrimoxazole (sulphamethoxazole and trimethoprim) since most patients are given cotrimoxazole before or during ART. The procedure started with extraction of nevirapine in which 100µl of patient plasma samples containing spike of nevirapine was transferred into polypropylene tube of 4ml and 25µl of carbamazepine (internal standard) with concentration of 11.6µg/ml was added followed by 200µl of carbonate buffer at pH 9.8. The resulting mixture was vortex mixed for 20 seconds and the drugs were extracted from plasma samples using 500µl of di- isopropyl ether by gentle shaking on reciprocal shaker for 10 min. After centrifugation for 10min at 3500rpm, 350µl of upper organic phase was transferred into another similar labeled polypropylene tube and dried by using a gentle stream of nitrogen gas at room temperature. The dried samples were reconstituted with 120µl of mobile phase and vortex for 3seconds. The content was transferred into auto sampler vials and 90µl of solution was injected into the chromatograph. Each run of patient test samples included quality control and standard curve samples. The standard curve samples were prepared by duplicate analysis of 100µl plasma spike with working solution of nevirapine to achieve concentration 8.6µg/ml. Three quality control samples (low, medium and high) containing nevirapine at the concentration of 2.58, 4.30 and 6.02µg/ml respectively were always included in each analysis. The resulting peak area ratios between the internal standard (carbamazepine) and analyte (nevirapine) were plotted versus concentration to obtain a linear regression equation (model), which was used to calculate nevirapine concentration in patient test samples. For higher nevirapine plasma concentrations a prepared dilution of 1:10 was performed. Nevirapine plasma concentration > 3µg/ml was categorized as good adherence [33]

Figure 1: A Chromatogram of blank plasma obtained after analysis of plasma sample collected from a volunteer.

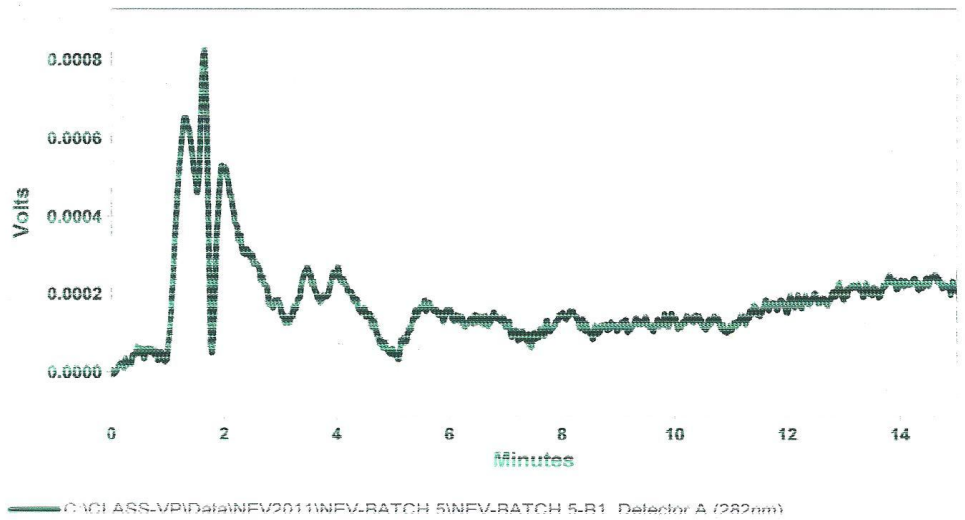
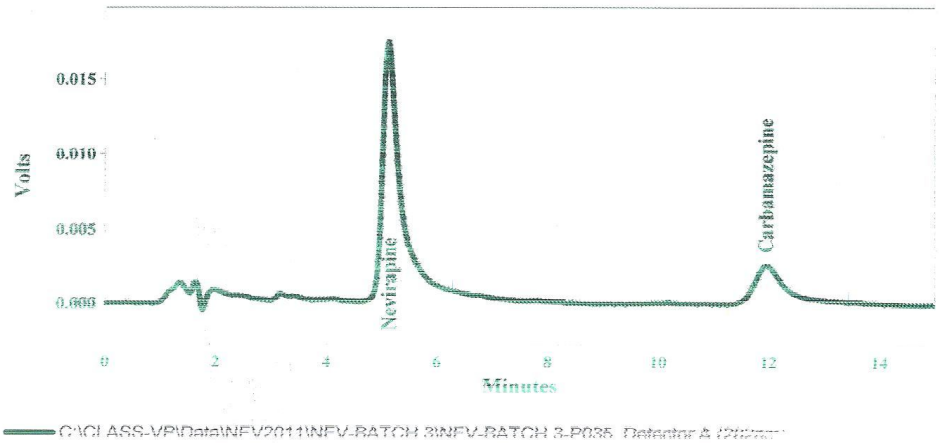


Figure 2: Chromatogram showing peaks of nevirapine and carbamazepine and their retention times from plasma of one HIV infected child undergoing ARV



6.10.0 Ethical clearance and consideration

6.10.1 Ethical clearance

Ethical clearance was sought from MUHAS research and publication committee. Permission to conduct the study at Mwananyamala, Temeke and Amana CTC was obtained from district medical officer DMO of Kinondoni, Temeke and Ilala respectively.

6.10.2 Ethical consideration

Prior to recruitment of participants to the study parents or caretakers of eligible patients were asked to give a written informed consent for their child's participation into the study. This was done after explaining the purpose of the study, procedures, benefits and potential risks or inconveniencies of participating in the study. They were informed that the finding of this study will help to improve the quality of care and treatment of HIV children .Parents or caretakers were provided with opportunity to ask question prior to consenting and at the end of each data collection session. They were informed on voluntary nature of participating and that they could opt out at the beginning or at any time during the study without penalty or loss of any right of the child or care taker. An assent was also sought from the older children before participating into the study. Confidentiality was ensured through use of ID code to conceal their identity. Unauthorized individuals were not allowed access to data collected which was password protected on a computer. For those who refuse to participate into the study, assurances were given that they will continue to receive the routine medical services as provided by facility. Requesting for permission to access medical records were part of consent. At the end of the session participants continued with activities of scheduled visit.

6.11 Data quality control

Data were checked daily for completeness, cleared, edited, coded and double entered in EPIDATA and then were exported to SPSS version 15 for analysis. Back up of data were done Filled questionnaires were stored in safe place.

6.12 Statistical analysis

6.12.1 **Univariate Analysis**

Mean and standard deviations were calculated for continuous variables. Histograms and pie chart were used to display the results. Percentages were calculated for categorical variables. Proportion of children with good adherence was calculated by taking number of children with good adherence divide by study sample size and confidence intervals were calculated.

6.12.2 Bivariate analysis

This was done to determine association between adherence as main predictor and other factors as potential confounders while CD4 count as an outcome. All continuous variables were categorized and analysed using chi square test or Fisher's exact test. $P < 0.05$ was considered significant. Odd ratio (OR) was the measure of association and confident intervals (95% CI) were reported.

6.12.3 Multivariate Analysis

Factors with p-value 0.2or less at bivariate analysis were selected for further multivariate analysis and entered in logistic regression model. This was done to assess the independence and strength of association of predictive factors of interest using CD4 count as outcome variable. It was further used to control for possible confounders of the main predictor and outcome. A difference of at least 10% between adjusted odd ratio and the crude odd ratio was considered confounding.

6.12.4 Kappa statistic

Kappa statistic was used to assess agreement between adherence measures (caretaker report, medication return and nevirapine plasma concentration).Kappa value range between -1 (complete disagreement) to +1 (complete agreement).

6.12.5 Sensitivity, Specificity, Positive predictive value and Negative predictive value

These statistical measures were used to measure validity and precision using CD4 count/ percentage to compare nevirapine plasma concentration, medication return and caretaker report to detect adherence and non adherence.

7.0 RESULTS

7.1.0 Description of study participants.

A total of 313 patients were screened for eligibility, 13 patients were excluded for various reasons including not being on nevirapine based regimen (5), on nevirapine based regimen less than 6 month (3), parent/caretaker not granting consent (2), severe malnutrition (3). A total of 300 participants met inclusion criteria were recruited into the study most of whom were above five years (81.3%) versus below five years of age(18.7%) with the mean age(SD) of 8(3) years . Of the children who participated in the study almost half were female (50.7%) (Figure1).

Majority (77%, n=231) had advanced stage of HIV infection and almost half were malnourished (Table 2). Of 176 children assessed for disclosure only (23.9%, n=42) knew their HIV status. Most of children (90.7%, n=272) were on ARV for more than a year and (31.7%, n=95) children had infections. More than three quarters (77.7%, n=233) of children were immunosuppressed at baseline while only (28%, n=84) were immunosuppressed when current CD4%/ count was checked (Table1)

Figure 3: Gender distribution of HIV infected children (N=300)

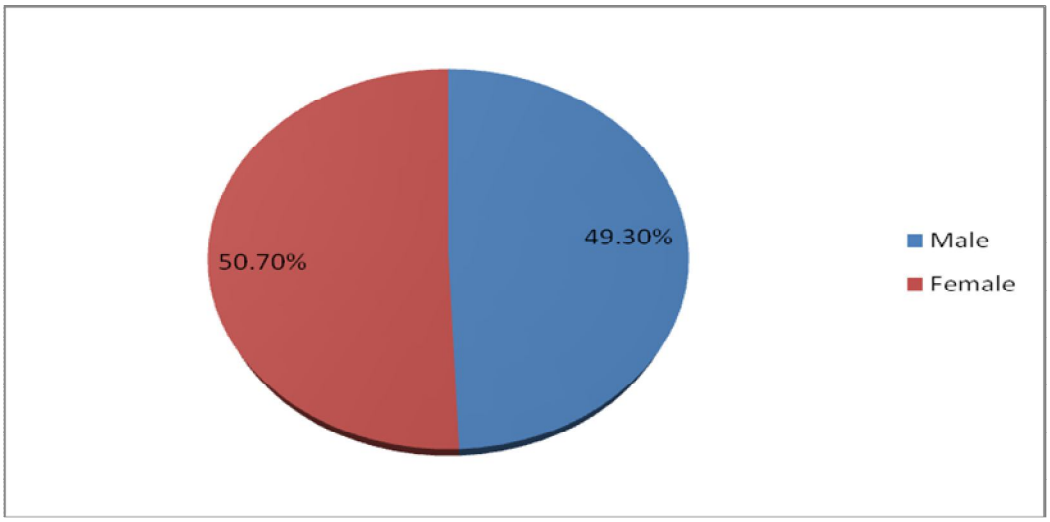


Table 1: Clinical characteristics of children

Variable	Frequency	Percentage
WHO Stage		
III and IV	231	77.0
I and II	69	23.0
Nutrition status		
Malnutrition	158	52.7
Normal	142	47.3
Disclosure of HIV status		
No	134	76.1
Yes	42	23.9
Duration of ARV		
≤1 year	28	9.3
>1 year	272	90.7
Overall Infection*		
Yes	95	31.7
No	205	68.3
Baseline CD4%/count		
Immunosuppressed**	233	77.7
Normal immunity***	67	22.3
Current CD4%/ count		
Immunosuppressed	84	28.
Normal immunity	216	72.

* Include oral candidiasis, skin lesion, otitis media, pneumonia,

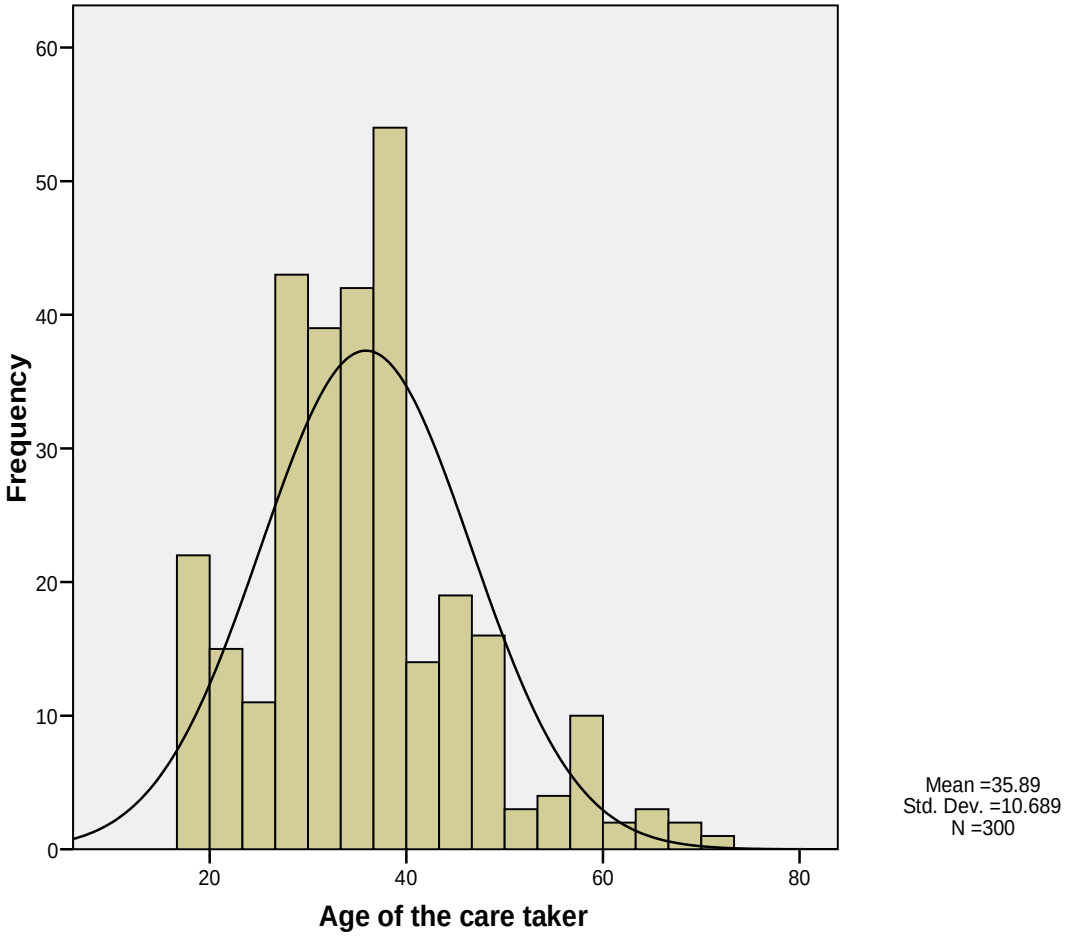
**** < 30%/500cell/ml**

***** >30/500cell/ml**

7.1.1 Socio-demographic characteristics of caretakers

The mean age of caretaker was 35.89± 10.69 years. Their ages were normally distributed; youngest caretaker was 18 years old, while the oldest was 70 years as shown in figure 2 below show distribution of caretaker by age.

Figure 4: Age distribution of caretakers



Of 300 caretakers (88.3%, n=265) were female and only (21%,n=63) of caretaker were educated beyond primary school, many of caretaker had primary education (68.7%,n=206). The majority of caretaker were Muslims (62.3%, n= 187) and most (58.7%, n=176) were mothers of children. 0f the 300 caretakers (36%n=138) were married and only (16.7%n=50) were employed (Table 2).

Table 2: Socio demographic characteristics of the caretakers (N=300)

Variable	Frequency	Percentage
Sex		
Male	35	11.7
Female	265	88.3
Education		
No formal education	31	10.3
Primary Education	206	68.7
Secondary education	54	18.0
Tertiary education	9	03.0
Occupation		
Business	132	44.0
Employed	50	16.7
House wife	91	30.3
Peasant	03	01.0
Student	24	08.0
Religion		
Christians	113	37.7
Muslims	187	62.3
Relation		
Mother	176	58.7
Father	21	07.0
Aunt	36	12.0
Sister	32	10.7
Grandparent	25	08.3
Brother	08	02.7
Uncle	02	00.7
Marital Status		
Married	138	36.0
Single	99	33.0
Divorced	20	06.7
Widow	41	13.7
Cohabiting	02	00.6

7.2.0 Proportion of good adherence measured by nevirapine plasma concentration, medication return and caretaker report.

Of 300 participants (85%, n=254) had good adherence assessed by nevirapine concentration while (15%, n=46) had non adherence. The proportion of good adherence assessed by medication returned and caretaker report were (97%, n=291), (98%, n=295) respectively. The confidence interval and p value were calculated using Z- test .The Table below has more detail.

Table 3: Proportion of good adherence to ARV among HIV children

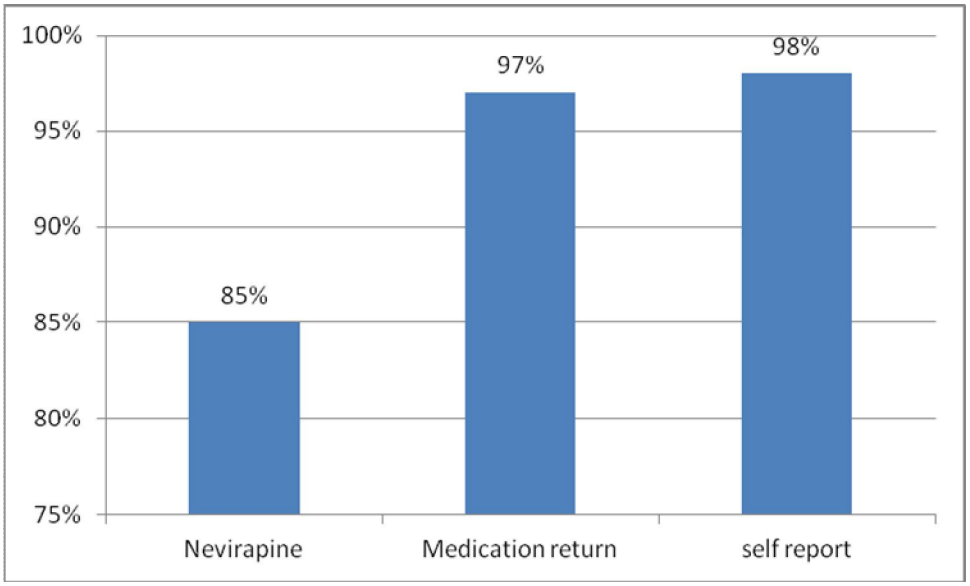
Variable	Frequency (N)	Proportion	95 % CI*	p-value
1 .Medication return				
≤5%	291	0.97	0.94-0.98	0.0001
>5%	9	0.03		
2.Caretaker self report				
Overall				
Never missed	295	0.98	0.96-0.99	0.0001
Missed	5	0.02		
3.Nevirapine conc				
≥3µg/ml	254	0.85	0.80-0.88	0.0001
<3µg/ml	46	0.15		

* Confidence interval.

7.3.0 Comparison of proportional of good adherence across adherence measures

The proportion of good adherence assessed by nevirapine plasma concentration was statistically significant lower (85%) when compared to medication returned (97%) p<0.0001 and caretaker report (98%) p<0.0001 respectively. There was no significant difference in proportion of good adherence between caretaker report and medication return (p=0.06) as shown in Figure 4 below.

Figure 5: Proportion Comparison of adherence



7.3.2 Agreement between adherence measures

Agreement between nevirapine plasma concentration and medication return and between nevirapine plasma concentration and caretaker report were weak (k=0.01) (p= 0.77), (k=0.13) (p=0.002) indicated in the Table 4 below. Agreement between medication return and caretaker report was also weak (k=0.12) (p=0.036).

Table 4: Agreement between nevirapine plasma concentration versus medication return and caretaker report

Variables	Nevirapine plasma concentration <3µg/ml	Nevirapine plasma comcentration ≥3µg/ml	Kappa value (95 %CI*)	P-value
Medication return				
> 5%	5	5	0.131(0.007-0.011)	0.002
≤5%	41	249		
Caretaker report				
Missed	1	4	0.01(1-1)	0.77
Not missed	45	250		

*Confidence interval

7.4.0 Association between adherence measures and immune status.

Adherence measures include caretaker report, medication return and nevirapine plasma concentration. Nevirapine plasma concentration < 3µg/dl was statistically significant associated with immunosuppression (OR=2.84, p=0.002).Caretaker report based adherence was not associated with immunosuppression (OR=0.64, p=0.569). Medication return was also not associated with immunosuppression (OR =1.75, p=0.474) as shown in (Table7)

Table 5: Bivariate analysis of Adherence measures and immune status (N=300).

Variable	Immune status Immunosuppression n(%)	Normal n(%)	OR	CI	P Value
1.Caretaker report					
Missed	1(20.0)	4(80.0)	0.64	0.07-5.79	0.569
Not missed	83(28.1)	212(71.9)	1		
2.Medication return					
> 5%	4(40.0)	6(60.0)	1.75	0.48-6.36	0.474
≤ 5%	80(27.6)	210(72.4)	1		
3.Niverapine plasma Concentration					
<3µg/ml	22(47.8)	24(52.2)	2.84	1.49-5.41	0.002
≥3µg/ml	62(24.4)	192(75.6)	1		

7.4.1 Measures of validity of nevirapine plasma concentration, caretaker report and medication return

Compared with immune status (CD4 count/percentage), sensitivity of nevirapine plasma concentration for detecting inadequate adherence was high 11.1% compared to medication return and caretaker report 4.7% and 1.2% respectively . See the details in Table 6.

Table 6: Measures of Validity (Sensitivity, Specificity and Predicative Value)

Variable	Immune status Immunosuppression n (%)	Normal n (%)	Sensitivity	Specificity	Positive Predicative value	Negative Predicative value
1.Caretaker report						
Missed	1(20.0)	4(80.0)	1.2%	98.2%	20%	71.8%
Not missed	83(28.1)	212(71.9)				
2.Medication return						
>5%	4(40.0)	6(60.0)	4.7%	97.2%	40%	72.4%
≤5%	80(27.6)	210(72.4)				
3.Niverapine plasma Concentration						
<3µg/ml	24(52.2)	22(47.8)	11.1%	73.8%	52.2%	24.4%
≥3µg/ml	192(75.6)	62(24.4)				

7.4.2 Other factors associated with immune status

Other factors considered as potential confounder were socio demographic factors of the child and caretakers, clinical factors (infections, nutrition status, disclosure, duration of ARV use and WHO clinical stage for HIV infection).

7.4.2.1 Socio- demographic characteristics and immune status

Children aged (2-5 years) were statistically significant (OR=5.52 CI 2.8-10.6) more likely to be immunosuppressed than those above 5years of age. The female children were (OR= 0.96, CI 0.58-1.59) less likely to be immunosuppressed but not statistically significant. Socio-demographic factors of care taker were not statistically significantly associated with immune status (Table 9)

Table 7: Bivariate analysis of socio-demographics and immune status (N=300).

Variable	Immune status		OR	CI	P Value
	Immunosuppressed n (%)	Normal n (%)			
Age of the child					
2-5years	33(58.9)	23(41.1)	5.52	2.8-10.6	0.001
6-8	18(21.4)	66(78.6)	5.26	2.5-11.08	
9-14	33(20.6)	127(79.4)	1		
sex of the child					
Male	42(28.4)	106(71.6)	1		
Female	42(27.6)	110(72.4)	0.96	0.58-1.59	0.898
Age of the care taker					
<45	74(30.3)	170(69.7)	1		
≥45	10(17.9)	46(82.1)	2.00	0.96-4.18	0.070
Sex of the care taker					
Male	7(20.0)	28(80.0)	1		
Female	77(29.1)	188(70.5)	0.61	0.26-1.46	0.320
Education of the care taker					
No education and Primary education	70(29.5)	167(70.5)			
≥Secondary education	14(22.2)	49(77.8)	1.47	0.76-2.83	0.273
Relation					
Parent	61(31.0)	136(69.0)	1		
Other relatives	23(22.3)	80(77.7)	1.56	0.89-2.71	0.136
Religion					
Christian	35(31.0)	78(69.0)	1		
Muslim	49(26.2)	138(73.8)	1.26	0.76-2.12	0.426
Marital status					
Married	41(29.7)	97(70.3)	1		
Single	28(28.3)	71(71.7)	1.07	0.61-1.89	0.811
Separated	15(23.8)	48(76.2)	1.35	0.68-2.68	0.388

7.4.2.2 Clinical factors and immune status

Children with infections were significant (OR=2.00, 1.18-3.37) more likely immunosuppressed than those without infection. Likewise children on ARV for less than 1year were significantly (OR=6.27, CI 2.69-14.63) more likely immunosuppressed than those on ARV for more than 1 year. Individual disease malaria, pneumonia, Otitis media, oral lesion and skin lesion were (OR=2.09, CI= 0.75-5.80), (OR=1.01, CI= 0.53-1.90), (OR=2.21, 0.66-7.46), (OR=1.46, CI=0.47-4.48) and (OR=2.74, CI=0.99-7.55) respectively more likely immunosuppressed but these were not statistically significant. Other clinical factors not significantly associated with immune status were nutrition status, disclosure and WHO clinical stage (OR=0.76, CI= 0.46-1.25), (OR=1.44, CI=0.58-3.57) and (OR=1.2, CI= 0.67-2.32).Table10.

Table 8: Bivariate analysis of clinical factors and immune status

variable	Immune status Immunosupression n(%)	Normal n(%)	OR	CI	P Value
Overall Infection					
Yes	36(37.9)	59(62.1)	2.00	1.18-3.37	0.013
No	48(23.4)	157(76.6)	1		
Malaria					
Yes	7(43.8)	9(56.3)	2.09	0.75-5.80	0.160
No	77(27.1)	207(72.9)	1		
Pneumonia					
Yes	16(28.1)	41(71.9)	1.01	0.53-1.90	0.999
No	68(28.0)	175(72.0)	1		
Otitis media					
Yes	5(45.5)	6(54.5)	2.21	0.66-7.46	0.189
No	79(27.30	210(72.4)	1		
Oral Lesion					
Yes	5(50.0)	6(54.5)	1.46	0.47-4.48	0.546
No	79(27.3)	210(72.7)	1		
Skin Lesion					
Yes	8(50.0)	8(50.0)	2.74	0.99-7.55	0.081
No	76(26.8)	208(73.2)	1		
Nutrition status					
Malnutrition	40(25.3)	118(74.7)	0.76	0.46-1.25	0.304
Normal	44(31.0)	98(69.0)	1		
Disclosure					
No	30(22.4)	104(77.6)	1.44	0.58-3.57	0.519
Yes	7(16.7)	35(83.3)	1		
WHO Stage					
iii and iv	67(29.0)	164(71.0)	1.25	0.67-2.32	0.540
i and ii	17(24.6)	52(75.4)	1		
Duration of ARV					
≤1yrs	19 (67.9)	9(32.1)	6.72	2.90-15.6	0.000
> 1yrs	65(23.9)	207(76.1)	1		

7.4.3 Multivariate analysis of the factors associated with immune status

This was done to assess the independence and strength of association of predictive factors of interest using CD4 count as outcome variable. Variable considered for multivariate were those with p-value < 0.2 at bivariate analysis. These variable included age of the child, sex of the care taker, relation to child, overall infection, malaria, otitis media and skin lesion. A difference of at least 10% between adjusted odd ratio and the unadjusted odd ratio was considered confounding. The final multivariate analysis was performed by backward elimination procedure taking into account the biological knowledge about independent variables and how they relate with the immune status. The strength of association after controlling for confounders nevirapine plasma concentration <3µg/ml was independently associated with two times (OR=2.47, CI=1.15-5.33) more likelihood of immunosuppression compare to those with nevirapine therapeutic level. Duration of ARV less than a year was independently associated with immunosuppression (OR= 6.79.CI=2.69-17.16). Other factors were not significant associated with immune status.The potential confounders of nevirapine plasma concentration were children with young age (2-5years) (OR= 6.00, CI =2.96-12.17) and infections (OR=2.00, CI=1.01-3.41) (Table 9)

Table 9: Multivariate analysis of the factors associated with immune status (N=300).

Variables	OR (95%CI*)	P-value
Nevirapine plasma conc		
<3µg/ml	2.47(1.15-5.33)	0.021
≥3µg/ml		
Age of the child		
2-5years	6.00(2.96-12.17)	0.0001
6-8	5.80(2.60-12.94)	0.0001
9-14	1	
Overall Infections		
Yes	2.00(1.01-3.41)	0.05
No	1	
Duration of ARV		
≤1year	6.79(2.69-17.16)	0.0001
>1year	1	

OR- Odd Ratio * Confidence interval

8.0 DISCUSSION

Adherence to Antiretroviral therapy is very crucial in order to maximize the benefit of the drugs. The benefit of ARV includes increase survival, improvement of immunity, prevention of development of opportunistic infections, improve quality of life, and promotion of growth and development. Inadequate adherence is associated with immunological, virological failure, drugs resistance and treatment failure.[24] The objective of this study was to determine proportion of good adherence and its association with immune status. Three type of measure were used to assess adherence include caretaker report, medication return and nevirapine plasma concentration.

8.1 Proportional of good adherence and comparing adherence measures

This study showed high proportion of children with good adherence to antiretroviral drugs by medication return (97%) and caretaker report (98%).

Despite the good adherence, it was found that adherence measured by nevirapine plasma concentration was significantly lower (85%) than medication return and caretaker report. In addition when assessment of agreement between the adherence measures were performed it was noted that there was a weak agreement between nevirapine plasma concentration versus care taker report (kappa 0.09) and medication return (kappa 0.131) respectively. This means that many of the children who were noted as if were adhering to high extent to ARVs when using the care taker report and medication return were found by corresponding plasma nevirapine concentrations obtain from the blood of the same children to have non adherence.

Generally non adherence by nevirapine was found to be 15% which is alarming when considering the management of HIV and AIDs in children. This means that approximately in every ten (10) children two (2) are not adhering to the ARV medication. However plasma level can be affected by adherence, drug quality and metabolic capacity of patient or vomiting after drug administration. It is unlikely that low plasma concentration in children was due to bioavailability of the drug formulation

or vomiting after drug intake because as it can be seen in Table 5 there is mixture of children with high and low nevirapine plasma concentration and none of the child was reported to vomit the medication. On the other hand metabolic capacity among individual children with respect CYP 3A and CYP 2B6 (major metabolizing enzyme) of nevirapine and efavirenz could impact plasma concentration even in children with good adherence. However a recent study conducted in Tanzania population indicates that the frequencies of hyplotype expressing increased activity of these enzymes are low among Tanzanian populations. [12] Therefore it is unlikely that the children showing sub therapeutic (15%) were extensive metabolizer. [12]This finding are in agreement with those obtain in Cameroon in which 11.3% of patients were found to have sub therapeutic nevirapine level (< 4µg/ml) considered non adherent to ARV. [34] . The small discrepancy between the results of these three studies might be explained by difference in age, study design and the different cut-off points for therapeutic nevirapine level 4µg/ml and 3µg/ml.

On the other hand, when looking at non adherence using medication return and care taker report our study has found that only 3% of the children are not adhering to ARV medication meaning that among one hundred (100) children three (3) were not adhering. This higher figure is similar to the study in Kampala in which the level of adherence assessed using medication return and caretaker reporting were 94%, 89% respectively. [29] Our finding advocates the importance of using drug plasma concentration in determining adherence which is objective way and superior to caretaker report and medication return.

8.2 Association between adherence measures and immune status

Nevirapine plasma concentration seems to give a better estimation of adherence to ARV than caretaker report and medication return. This study found that nevirapine plasma concentration was strongly associated with immunosuppression (p=0.021) while medication return >5% was not associated with immunosuppression (P=0.474) .This finding was similar to study conducted in South Africa where medication was not associate with immunological response (P=0.075) [30]

In contrast to nevirapine plasma concentration we found that caretaker reported missed doses was also not associated with immunosuppression (p= 0.569). These finding indicates nevirapine plasma concentration could be a good predictor of immune status while caretaker report and medication return are poor predictor of immune status.

Furthermore we found that caretaker report and medication return have low sensitivity to detect non adherence children compared to nevirapine plasma concentration method which could detect bigger proportion of non adherence children. At the moment, medication return and caretaker report, have been adopted as methods for measuring adherence in children undergoing ART by all paediatric HIV clinics in Tanzania. This means that, a great deal of non-adherent children are being managed unnoticed and this has clinical implication in terms of treatment outcomes and drug resistance. The detection of non adherence is very important in order to intervene before development of drug resistance and treatment failure. Intervention to improve adherence have been advocated includes knowledge and counselling, social support, financial incentive and technological device or remainder example phone, watch and alarm.

8.3 Other independent factors associated with immune status

Children with infections were found to be independently associated with immunosuppression. Children with infection were more likely to be immunosuppressed than those without infection. These infections include pneumonia, otitis media, skin infection, oral candidiasis and malaria. HIV infected children are at risk of developing opportunistic infection because human immunodeficiency virus cause progressive destruction of CD4 cell which fight against infections, reduced number of CD4 cell result in development of opportunistic infection. Furthermore immunosuppressant occurs when they have infection so it is vicious cycle. Children with opportunistic infection have low CD4 value compared to children without opportunistic infection as has been reported with other study. [43]

The duration of ARV use was found to be associated with immune status, those children on ARV less than 1year were more likely to be immunosuppressed than the one on ARV

more than 1year.The explanation to this finding might be the duration of ARV was too short to improve CD4 to normal level and most of children may have had severe immunosuppression at initiation of ARV. Similar finding have been reported from USA where the effect of ARV on CD4 percentage/ count was investigated. It was found that the CD4 percentage after 1year of ARV in severe immunosuppressed children increase by 4.44% and slowly progressively increase thereafter. However, children with severe immunosuppression at baseline did not recover to normal immunity even after 5year of ARV treatment.[48]

Furthermore in this study the age of the child was independently and strongly associated with immune status; children with young age were six time more likely to be immunosuppressed than older children. This finding may be explained in terms of maturity of immune system. Young children have immature inexperienced immunity to fight against infections and in addition HIV infection cause further deterioration of CD4 value in young children compare to older children. [49]

On the other hand this study found that mild and moderate malnutrition was not associated with immunosuppression while study in India found that increase severity of malnutrition was correlated with decrease in CD4 count.[41] Severe malnutrition lead to depletion of CD4 count and this might be aggravated with HIV infection. [42] Malnutrition in children cause wide spread of atrophy of lymphoid tissue include thymus, spleen tonsil and lymph nodes, evidence of atrophy is greatest in T-lymphocyte area of these tissue. The disparity in finding is likely to be due to exclusion of patients with severe malnutrition in this study and this may explain why the effect of malnutrition on CD4 count could not be detected. Socio demographic factors of caretaker were also not associated with immune status. This is probably due to lack of direct effect of caretaker socio demographic factors on immune status of children.

9.0 LIMITATIONS OF THE STUDY

- The nature of the design of this study does not allow for assessment of causal relationships among the variables of interest and thus strong conclusions cannot be drawn. Time and other resource constraints determined the choice of the current design. A prospective cohort study would generate data that could be used to make causal inference.

- Caretaker report of adherence is subject to recall bias and this might lead to overestimating adherence. This was minimised by asking for number of doses taken on each day in previous 3day prior interview.

- The genotyping of children with respect to metabolising enzyme-CYP 2B6 and CYP 3A was not done to determine the metabolic capacity of individual children despite that a recent study conducted in Tanzania population indicates that the frequencies of hyplotype expressing increased activity of these enzymes are low among Tanzanian populations[12]

- Despite these limitations this study assessed adherence by using drug level which is objective method. This might give better estimation of proportion of adherence to ARV in HIV infected children and findings may be valid to be generalised to target population.

10.0 CONCLUSIONS

- Proportion of adherence assessed by caretaker report and medication return was higher however; proportion of adherent children assessed by nevirapine plasma concentration was significantly lower than caretaker report and medication return.

- Nevirapine plasma concentration has higher sensitivity to detect non adherence compare to caretaker report and medication return. Non adherence by nevirapine plasma concentration was high 15% which is alarming when considering management of HIV infected children.

- Nevirapine plasma concentration is a good predictor of adherence and correlate with well immunosuppression but caretaker report and medication return were poor predictors of adherence and did not correlate with immunosuppression.

11.0 RECOMMENDATIONS

- Nevirapine plasma concentration should be used to assess adherence in HIV infected children who develop clinical, immunological and virological failure.

- We recommend qualitative studies to explore the reasons for poor correlation between nevirapine plasma concentration and caretaker reported adherence as well as medication return and reasons for non adherence in paediatrics HIV care. Further studies also are needed to determine factors lead to low nevirapine plasma concentrations.

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13.0. APPENDIXES

13.1. Appendix I: QUESTIONNAIRE

1. ID number of the child.....
2. Interviewer number.....
3. Name of facility.....
4. Date of interview.....
5. Age.....
6. Sex.....
7. A)CD4 count/ % .....B) nevirapine plasma conc.....mg/dl

SOCIAL DERMOGRAPHIC INFORMATION ON INFORMANTS / CARETAKER

8. Sex.....
9. Age.....
10. Education level a) No education
    b) Primary education
    c) Secondary education
    d) Tertiary Education

11. Employment status
    a) Business
    b) Employed c) house wife
    d) Peasant e) Student
    f) Other.....

12. Relationship with the child
    a) Mother
    b) Father

c) Aunt

d) Other.....

13. Religion a) Christian b) Muslim c) other.....

14. Marital status a) Married b) Single c) Divorced d) Cohabiting
e) Others.....

15. When the child was first diagnosed? (Age-month.....years.....

16. For how long the child is on antiretroviral therapy? Month.....years.....

17. Which medication is the child on/ Check on the card? a) AZT, 3TC, NVP b) d4T, 3TC, NVP c) ABC, 3TC, NVP d) Others.....

18. Did the child suffer from any illness since last visit? a) YES b) NO. If yes go to question no 19.

19. IF YES a) malaria b) pneumonia c) Otitis media d) diarrhoea e) other.....

20. a) Is the child sick today? a) YES b) NO. If yes go to question no 21

21. IF YES a) Fever b) cough c) diarrhoea d) ear discharge e) oral thrush f) other.....

22. Does the child know his/her HIV status? a) YES b) NO.....

23. A)Anthropometric measurement

Weight	Kg
Height/ length	Cm
MUAC	Cm
Head circumference	Cm

Conclusion. a) Normal b) mild malnutrition c) moderate malnutrition

B) General examination and physical examination

finding.....

.....

.....

Clinical diagnosis a) pneumonia b) otitis media c) oral candidiasis c) malaria d) skin infection

d) others.....

C)Treatment.....

.....

.....

ASSESSMENT OF ADHERENCE AND NON ADHERENCE

24. i) Do you have medication with you? May I see them? Medication returned (amount).....

a) ≤5% of prescribed dose b) >5%of prescribed dose

ii) The dose per day.....

25. Please can you tell me when you give child medication? a) Morning

b) Afternoon c) Evening d) Morning and evening

e) others.....

26. How many doses did the child miss taking yesterday? a) None

b) One dose c) Two dose

27. How many doses did the child miss taking day before yesterday?

a) None b) One dose c) Two dose

28. How many doses did the child miss taking 3 days ago?

a) None b) one dose c) two dose.

29. Are there any other medications the child is taking? a)YES b)NO If yes go to question no 30
30. IF YES which medication a) cotrimoxazole b) amoxicillin c) ant TB d) other.....

31. I know it is difficult to take medication on daily basis, if you sometime miss a dose please tell me what causes this to happen? a) Simple forgetful b) we travel c) child slept d) caretaker away e) bad taste
g) other.....

32. Can you tell me what assisted you to give child medication on time and regularly? a) Clock b) individual c) others.....

33. Did antiretroviral therapy have been changed since the child started on ARV? a)YES b)NO. If yes go to question no 34

34. If YES why a) adverse reaction b) treatment failure c) other

13.2. Appendix II: DODOSO

1. Namba ya utambulisho ya mtoto.....

2. Namba ya udahili.....

3. Jina la kituo cha Afya.....

4. Tarehe ya udahili.....

5. Umri wa mtoto.....

6. Jinsia ya mtoto.....

7. a) CD4 Count/ %age.....b) Nevirapine plasma conc.....mg/dl

MZAZI/ MLEZI

8. Jinsia.....

9. umri.....

10. Elimu
a) sijasoma
b) s/msingi
c) sekondari
d) elimu ya juu

11. Kazi
a) mfanya biashara
b) mfanyakazi wa mshahara
c) Mama wa nyumbani
d) mkulima
e) mwanafunzi
f) nyinginezo.....

12. Uhusiano na mtoto

a) mama

b) baba

c) shangazi

d) dada

e) nyinginezo.....

13. Dini a) mkristo

b) mwisilamu

c) nyingine.....

14. Hali ya ndoa a) nimeoa/ nimeolewa

b) Sijaoa/sijaolewa

c) tumetengana

d) nyingine.....

15. Mtoto aligunduliwa ana VVU akiwa na umri gani? Miezi.....Miaka.....

16. Mtoto ametumia dawa kwa muda gani? Miezi.....Miaka.....

17. Mtoto yuko kwenye dawa gani? Angalia kadi a) AZT,3TC,NVP b) d4T,3TC,NVP c)ABC,3TC,NVP d) nyinginezo.....

18. Mtoto alikuwa anaumwa alipokuja klinic mara ya mwisho? a) Ndiyo b)Hapana. Kama ndiyo

nenda swali namba 19

19. Kama Ndiyo a) malaria b) pneumonia c)otitis media d) kuharisha

e)mengineyo.....

20. Mtoto anaumwa leo? a) Ndio b) Hapana. Kama ndiyo nenda swali namba 21

21.Kama ndiyo a) homa b) kukohoa c) kuharisha d) masikio yanatoa usaha e)mengineyo.....

22. Je mtoto anajua ana VVU? a) Ndiyo b) hapana

23. Vipimo

Uzito kg.....

Urefu cm.....

Mzunguko wa kichwa cm.....

Mzunguko wa mkono cm.....

a) Kawaida b) anupungufu kidogo wa lishe c) anaupungufu wa lishe

B) Uchunguzi wa mwili .....

.....

.....

D)Mtoto anaumwa a) nimonia b) otitis media c) oral candidiasis d) ugonjwa wa ngozi e) nyinginezo.....

MATUMIZI DHABITI YA DAWA

24.i)Umekuja na dawa? Naweza kuziona? Hesabu/pima dawa zilizobaki/iliyobaki.....

a) ≤ 5% ya dawa alizoandikiwa b) > 5% ya dawa alizoandikiwa

ii) Dozi yake.....

.....

25. Ni muda gani anameza dawa a) asubuhi b) mchanac) jioni d) asubuhi na jioni

26. Ni dozi ngapi za dawa mtoto alikosa kumeza jana? a) hajakosa b) moja c) dozi mbili

27. Ni dozi ngapi za dawa mtoto alikosa kumeza juzi? a) sikuwahi kukosa b) mara moja c) mara mbili

28. Ni dozi ngapi za dawa mtoto alikosa kumeza siku tatu zilizopita?

a) sikuwahi kukosa b) mara moja c) mara mbili

29. Kuna dawa zingine mtoto anakunywa?

a) ndiyo b) hapana . Kama ndiyo nenda swali namba 30

30. Kama ndiyo a) septrine b) amoxylini c) dawa za TB

d) nyingine. taja.....

31. Najua ni ngumu kunywa dawa kila siku ni sababu gani zinakufanya ushindwe kumpa mtoto dawa? a) kusahau. b) nikisafiri c) mtoto analala d) mlezi hayupo

e) ladha mbaya ya dawa f) nyingine taja.....

32. Nini kinakusaidia kukumbuka kumpa mtoto dawa kwa wakati kila siku? a) saa

b) mimi mwenyewe c) nyingine taja.....

33. Mtoto alishawahi badilishiwa dawa? a) ndiyo b) hapana .

34. Kama ndiyo a) zilinidhuru b) za mwanzo hazikunisaidia

c) nyingine taja.....

13.3. Appendix III: CONSENT FORM AND ASSENT

A) CONSENT

Study No.....

Title; Adherence to ARV and its association with immune status among HIV infected children in Dar es Salaam

To the parent/Caretaker.....

Foreword

I am Dr. Frida Mghamba a postgraduate student at MUHAS conducting a study on adherence to ARV and its association with immune status.

How to Participation

Parent/guardian/ caretaker will fill a questionnaire administered by the PI or research assistance. The child will be examined and finding will be documented in the questionnaire. Weight, height/ length and MUAC will be measured. Important finding will be documented in patient file and discussed with attending physician.

Purpose of study

The study will generate statistic on adherence to ARV in HIV infected children and its association with immune status, therefore will help to improve adherence to ARV in children. Good adherence will maximize the benefit of antiretroviral therapy and reduce mortality and morbidity among HIV-infected children.

Right to refusal or withdrawal

The participation is entirely voluntary and they are free to agree or disagree to participate in this study. They may withdraw from study at any time even after signing this form.

Risks

No risk is expected to occur to your child as result of participation in this study.

Confidentiality

Discussion with PI/ research assistance is confidential and your name will not appear anywhere on coded form with information. Paper and computer record s will be kept under lock and key and security code respectively.

Consent

I have understood the explanation of study. I accept for my child to be examined and participate in the study.

Signature of caretaker/ parent/guardian.....Date.....

Relationship to the child.....

Signature of interviewer..... Date.....

Thumb print.....

B) ASSENT for older children> 7years

Greetings; I'm Dr Frida Mghamba conducting a study to assess adherence to medication and its association with immune status, risk, benefit explained. The study involves examination and taking blood samples.

Child a) agree b) disagree

If agree to participate into the study

Signature of the child.....Date.....

Thumb print.....Date.....

For more information or clarification you may contact number below

DR Frida Mghamba , 0713296424

13.4. Appendix IV: FOMU YA RIDHAA

A) Ridhaa ya Mzazi/mlezi

Namber ya utafiti.....

Kichwa cha habari; Matumizi dhabiti ya dawa za virusi vya ukimwi na uhusiano wake na kinga ya mwili kwa watoto wenye virusi vya ukimwi Dar es Salaam.

Utangulizi

Mimi ni Dr Frida .W.Mghamba in mwanafunzi wa udhamili chuo kikuu cha sayansi za afya cha muhimbili . Ninafanya utafiti wa matumizi dhabiti ya dawa za virusi vya ukimwi na uhusiano wake n kinga ya mwili kwa watoto wenye virusi vya ukimwi (VVU).

Taratibu za Ushiriki

Mzazi/ Mlezi atashiriki katika dodoso litakalotolewa na mtafiti mkuu au msaidizi wake. Mtoto atapimwa na matokeo yatajazwa kwenye dodoso uzito, urefu na MUAC vitapimwa . Matokeo muhimu yatajazwa kwenye file.

Dhumuni la utafiti

Utafiti huu utatuwezesha kupata takwimu ya idadi ya watoto wenye virusi ambao wanatumia vizuri dawa za virusi vya ukimwi na kama ina uhusiano na kinga ya mwili. Takwimu zitatumika kuongeza matumizi ya dawa za vvu kwa watoto ili dawa zifanye kazi inavyotakiwa hivyo kupunguza vifo vya watoto.

Haki ya kukubali au kukataa

Mshiriki ana uhuru kamili wa kukubali au kukataa kushiriki kwenye utafiti huu. Pia unaweza kujitoa katika utafiti huu wakati wowote hata baada ya kusaini fomu hii.

Madhara

Hakuna madhara yanayotarajiwa kutokana na ushirika wa mtoto wako kwenye utafiti

Usiri

Taarifa yote inayokusanywa itaingizwa kwenye komputa kwa utambulisho wa numba tu.Hivyo ushirika wako hautaweza kutambuliwa.

Ridhaa ya makubaliano/ kukubali

Nimeelewa maelezo kuhusu utafiti huu. Nakubali mwanangu apimwe na kushiriki katika utafiti huu.

Saini ya mzazi/mlezi----------tarehe----------

Uhusiano wake na mtoto----------

Saini ya mdahili----------tarehe----------

Kidole gumba----------

B) Ridhaa kutoka kwa mtoto mwenye umri zaidi ya miaka 7

Habari yako; Mimi ni Dr Frida Mghamba nafanya utafiti wa matumizi dhabiti ya dawa, hakuna madhara yanayotarajiwa kutokea.Utafiti huu utahusisha vipimo kama damu, un haki ya kukubali au kukataa

Mtoto a) kakubali b) kakataa

Kama mtoto kakubali

Saini ya mtoto..... tarehe .....

Kidole gumba.....

Kwa ufafanuzi au maelezo zaidi waweza wasiliana kwa kutumia numba ifuatayo

Dr Frida Mghamba simu numba 0713296424

13.5. Appendix V: WHO IMMUNOLOGICAL CLASSIFICATION.

HIV- associated immunodeficiency	Age- related CD4 value		
	0 – 11 months (%)	12-59 months (%)	>5years (count/ %)
Not significant	>35	>30	>500
Mild	30-35	25-30	349-499
Advanced	25-30	20-25	200-349
Severe	< 25	<20	<200 or 15%