HEPATITIS B AND C VIRAL CO-INFECTIONS AMONG CHILDREN INFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS ATTENDING THE PAEDIATRIC CARE AND TREATMENT CENTER AT MUHIMBILI NATIONAL HOSPITAL, DAR-ES-SALAAM, TANZANIA

By

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dissertation submitted in partial fulfillment of the requirements for the degree of aster of Medicine (Paediatrics and Child Health) of the Muhimbili University of llied Sciences.

Muhimbili University of Health and Allied Sciences

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Muhimbili University of Health Allied Sciences, a dissertation entitled: ***Hepatitis B and C viral co-infections among children infected with Human Immunodeficiency Virus attending the Paediatric Care and Treatment Center (CTC) at Muhimbili National Hospital in Dar-es-salaam, Tanzania,*** in partial fulfillment of the requirements for the degree of Master of Medicine (Paediatrics and Child Health)

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DECLARATION AND COPYRIGHT

I, **Safila Philipo Telatela**, hereby declare that this dissertation is my own original work and it is a product of my own efforts and that it has not been presented and will not be presented in any other University for a similar or any other degree award.

Signature.....

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Glory and honor be to GOD and the Lord Almighty who was with me throughout this work and my studies at large. AMEN.

DEDICATION

To my beloved husband Mathias Ombeni, my son Prosper, my daughter Priscilla, my beloved parents Mr. & Mrs.Philipo Telatela.

ABSTRACT

Background: Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infections are becoming a cause for significant concern in HIV infected children.

Objective: To determine the seroprevalence and risk factors for HBV and HCV among HIV infected children aged 18 months to 17 years, attending paediatric HIV care and treatment center (CTC) at MNH, Dar-es-Salaam, Tanzania.

Study design and setting: A cross sectional study conducted at the paediatric care and treatment center of the Muhimbili National Hospital between April 2006 and August 2006.

Methodology: A standard structured questionnaire was used to obtain patient particulars. Blood was collected for examination of HBsAg, HCV Abs and ALT levels. HIV status and CD4 counts were obtained from patient records.

Results: A total of 167 HIV infected children, 88(52.7%) males and 79(47.3%) females were enrolled. The overall prevalence of hepatitis co-infection was 15%, with the prevalence of HBV and HCV co-infection being 1.2% and 13.8%, respectively. Hepatitis co-infection was not associated with any of the investigated risk factors. There was no association between HBV and HCV. Elevated ALT (level) was association hepatitis viral co-infection but not with ART or impairment in immune status.

Conclusion and recommendation: The high seroprevalence (15%) of hepatitis co-infection in HIV infected children attending the paediatrics HIV CTC at the MNH calls for routine screening of hepatitis viral co-infection. Notably, children with

hepatitis viral co-infection had significantly higher levels of ALT and lower counts of CD4+ lymphocytes.

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LIST OF ABBREVIATIONS

AIDS Acquired Immunodeficiency Syndrome

ALT Alanine Aminotransferase

ALP Alkaline phosphatase

ART Antiretroviral therapy

CBC Complete Blood Count

CHB Chronic Hepatitis B

DNA Deoxyribonucleic Acid

ELISA Enzyme-Linked Immunosorbent Assay

HIV Human Immunodeficiency Virus

HBV Hepatitis B Virus

HCV Hepatitis C Virus

HBsAg Hepatitis B surface Antigen

ART Highly Active Antiretroviral Therapy

LFT Liver Function Test

MTCT Mother-To-Child Transmission

MNH Muhimbili National Hospital

MOH Ministry of Health and Social Welfare

TDF Tenofovir Disoproxil Fumarate

UNAIDS United Nations Joint Programme on HIV/AIDS

USPHS/IDSA United States Public Health Service/ Infectious Disease Society of America

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Background information

The human immunodeficiency virus (HIV), the causative agent of acquired immune deficiency syndrome (AIDS), is a major cause of infant and childhood morbidity and mortality. By the end of 2006 UNAIDS estimated that about 39.5 (34.1 - 47.1) million people were living with HIV/AIDS in the world; of these 1.7 - 3.5 million were children under the age of 15 years. Sub Saharan Africa is the region most affected by the HIV/AIDS pandemic, where 24.7 (21.8 - 27.7) million (63%) people with HIV live. As HIV infection rates rise in the general population, new infections are increasingly occurring in younger age groups.[1]

In December 2006 the UNAIDS/World Health Organization (WHO) released statistics showing that 530,000 children under age of 15 years were newly infected with HIV and that three million children in sub-Saharan Africa were living with HIV.

More than 95 percent of the global total population of HIV-infected people now lives in developing countries, where 95 percent of all deaths from AIDS occur.[2] In seven sub-Saharan African countries, mortality due to HIV/AIDS in children under the age of five years has increased by 20 to 40 percent. [2]

1.1.1 Magnitude of HIV/AIDS epidemic in Tanzania

In Tanzania, the prevalence of HIV infection is estimated to be between 6.4% and 11.9%.[4] In 2003, the number of children (0 – 15 years) living with HIV/AIDS in Tanzania was estimated to be between 85,000 and 230,000. [1, 2] There are several

factors contributing to the high HIV prevalence in children in sub-Saharan Africa which include; high prevalence of infections in women of childbearing age, [4] high rates of mother to child transmission (MTCT) e.g. 40% in Botswana[1] and relative inaccessibility to Antiretroviral therapy (ART).[1] A large number of infected children have been associated with significant increase in morbidity, hospitalization and mortality.[4]

For example, the prevalence of HIV infection among children admitted at Muhimbili National Hospital in 1996 was found to be 19.2% and the mortality rate among these infected children was 21.4%.[3] It is estimated that 50% of infants infected perinatally die before their second birthday.[3]

1.1.2 HIV associated infections and illnesses

Like adults, children with HIV infection are prone to a variety of bacterial, viral, fungal and parasitic infections due to weakened immune defences.[4] However, in children, the immunosuppressive effects of HIV infection are additive to the immature immune system, making them even more vulnerable to infections than adults.[4]

Some of the common childhood infections and conditions experienced by HIV infected children include; diarrhoea, acute lower respiratory tract infections, septicaemia, acute suppurative otitis media, sinusitis, and failure to thrive.[1] In young infants, the earliest clinical signs and symptoms may be non-specific, such as failure to thrive, acute respiratory infections, and diarrhoea.[4]

With the advent of antiretroviral therapy (ART) and increased availability of antibiotics and antifungal agents, the incidence of opportunistic infections due to fungal and bacterial agents is significantly reduced.[5] On the other hand, co-infections with HBV and HCV are becoming a cause for significant concern.[5] These hepatitis viruses, which share the same routes of transmission with HIV, also influence the natural history and prognosis of the latter virus.[6] In addition, the co-infections have grave consequences, and pose potential challenges in treatment, including the potential for drug-drug reactions.[7] In a nutshell, hepatitis viruses, especially hepatitis B and C, are emerging as the leading causes of morbidity and mortality among children on ART,[8] warranting serious considerations of the presence of co-infections.

1.2 Literature Review

1.2.1 Hepatitis B and HIV co-infection

There is ample evidence indicating that human immunodeficiency virus (HIV)-positive individuals are more likely to be infected with hepatitis B virus (HBV) than HIV-negative individuals, possibly as a result of shared risk factors.[9] There is also evidence that HIV-positive individual who is subsequently infected with HBV is more likely to become HBV chronic carrier, have a high HBV replication rates, and remain HBeAg positive for a much longer period.[9]

Furthermore, HIV infection exacerbates liver disease in HBV co-infected individuals, and there is an even greater risk of liver disease when HIV and HBV co-infected patients are treated with anti-retroviral therapy (ART).[9]

1.2.2 Influence of HIV on the Course of HBV Infection

The course of acute hepatitis B infection may be modified in the presence of HIV with lower incidence of icteric illnesses and a higher HBV carriage rate of about 25% compared to about 5% in those uninfected with HIV.[10, 11] There is a trend towards lower rate of clearance of the hepatitis B "e" antigen (HBeAg) and HBV DNA as well as a significant increase in the serum HBV DNA viral load.[12,13] Additionally, HIV-induced immunosuppression may result in lower serum aminotransferases, possibly due to a reduction in the severity of liver disease.[14] However, immunosuppression may also be associated with reactivation of HBV infection in persons who have lost detectable HBsAg or HBeAg.[15,16] Although, symptomatic reactivation and loss of anti-HBs is uncommon in HIV-infected individuals,[16,17] asymptomatic reactivation or reinfection occurs frequently in patients who develop AIDS, leading to a significantly higher prevalence of HBsAg.[10] In a prospective cohort study it was found that serum HBV DNA levels were higher,[18] alanine aminotransferase (ALT) levels were lower and loss of serum HBsAg occurred at a lower rate in HIV carriers compared with HIV uninfected carriers (relative hazard, 0.39, CI 0.16-0.94).[20] Therefore, HIV seropositivity has been associated with significantly lower ALT levels, higher serum HBV DNA levels, lower rate of serum HBeAg and serum DNA clearance, decreased liver injury, and an increased loss of anti-HBs.[18]

1.2.3 Diagnosis of HBV

Virtually all individuals infected with HBV, either acutely or chronically, will have detectable serum hepatitis B surface antigen (HBsAg). In acute infections, HBsAg is detectable several weeks after the infection and its detection coincides with the onset of clinical symptoms. HBeAg is also detectable in acute infection, which is characterized by a high rate of viral replication. At around the same time, IgM antibodies against HBcAg are detectable in serum. Subsequently, IgG antibodies against HBcAg are produced. As the acute infection resolves, IgG antibodies against core antigen persist. IgM antibodies and HBsAg become undetectable. Subjects who develop an immune response against HBV also develop antibodies against HBsAg. Such antibodies can also be produced by vaccination. Most people who have had acute infection that resolves continue to have IgG antibodies against core antigen for life. Some remain immune with antibodies against HBsAg but some lose these antibodies and may be susceptible to future infection.[19]

Diagnosis and prognosis of HBV are determined by liver biopsy. Most people who are chronic carriers (no symptoms, HBsAg positive and normal serum aminotransferase activities) generally have little or no inflammation on liver biopsy analysis. In such patients, one can often see "ground glass cells" on liver biopsy which are liver cells in which large amounts of HBsAg are being synthesized. Other individuals with chronic hepatitis B will have various degrees of liver inflammation on liver biopsy analysis. Others will have fibrous or cirrhotic liver. The degree of inflammation, and the presence of fibrosis or cirrhosis, correlates with a worse prognosis.[20]

1.2.4 Management of patients co-infected with Hepatitis B Virus and HIV

The management of chronic hepatitis B virus (HBV) infection poses specific problems in the presence of HIV co-infection, since therapeutic approaches have to consider both HBV and HIV infections. There are currently four drugs approved for the treatment of chronic HBV infection: standard interferon alpha (Intron A), peginterferon α-2a (Pegasys), lamivudine (Epivir-HBV), adefovir (Hepsera), and entecavir (Baraclude). Other drugs include tenofovir and emtricitabine.

The dual antiviral activity of tenofovir and emtricitabine broadens the armamentarium against HBV in HBV-HIV co-infected patients. Nucleotide analogues (the nucleotide analogues adefovir and tenofovir have the advantage of a higher genetic barrier to the development of resistance compared to nucleoside analogues lamivudine and emtricitabine. Fortunately, the two groups of drugs do not share resistance mutations, allowing salvage therapy and the possibility of combination therapy for drug-naive individuals.

It is important to note that the response to standard interferon alpha is poorer in HBV-HIV co-infected patients compared to HIV-negative individuals. [21] This is especially true in hepatitis B e antigen-negative HBV infection [21]The more potent pegylated forms of interferon alpha are now available and have brought new hope for greater success with use of this immunotherapy.[21]

1.2.5 Hepatitis C and HIV co-infection

In the era preceding treatment of HIV infection with highly active antiretroviral therapy, HCV co-infection was of little concern because of the short-term survival of patients with HIV infection prevented the slowly developing consequences of chronic hepatitis C. As the life expectancy of patients with HIV infection increased with therapy, HCV has emerged to be a significant pathogen of concern. HIV-HCV co-infection has been associated with higher titers of HCV, more rapid progression to HCV-related liver disease, and an increased risk for HCV-related liver cirrhosis.[22] Because of this; HCV infection has been viewed as an opportunistic infection in HIV-infected persons. Based on that, HCV was included in the 1999 USPHS/IDSA Guidelines for the Prevention of Opportunistic Infections in Persons Infected with Human Immunodeficiency Virus.[23]

Several lines of evidence in adult patients suggest that liver disease may be more severe in patients co-infected with HIV. Progression of HIV disease may be accelerated by HCV co-infection, [24] whether co-infected children may share these clinical patterns it remains a matter of speculation. Chronic hepatitis C in otherwise healthy children is usually a mild disease. Liver damage may be sustained and fibrosis may increase over the years, suggesting slow progression of the disease.[23, 24]

Children requiring multiple blood transfusions due to cancer or other causes are at an increased risk for blood transmittable diseases such as hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) infection.[25] A study done at the Muhimbili National Hospital in 1999 by Kitundu *et al*[26] among

children with malaria-associated anaemia, the prevalence of HCV infection was 7.1%. However, there was no significant difference in the prevalence of HCV infection between transfused and non-transfused children and no risk factors could be found.

1.2.6 Influence of HIV on the course of HCV infection

HIV co-infection has been associated with a more rapid progression of liver disease as well as a higher prevalence of Liver cirrhosis.[42,43,44] In individuals with parenterally acquired HCV infection (blood transfusion recipients , there is a higher incidence of cirrhosis within the first 15 years of follow-up (15-25% vs 2.6-6.5%, respectively, ($p < .05$) in HIV-positive individuals compared to those who are HIV negative.[45,46] Additionally, the estimated interval from HCV infection to cirrhosis may be significantly shorter in the HIV-infected individuals (7 vs 23 years, $p < .001$).[45]

Although HIV/HCV co-infection results in a more rapid progression of liver disease, its effect on mortality requires further assessment. Some studies have found a higher rate of mortality from liver-related diseases in the co-infected patients[47, 48] yet others have not shown any effect on survival.[49, 50]

1.2.7 Diagnosis of HCV

The available assays for the detection of HCV infection are based on the detection of antibodies to HCV antigens or testing directly for viral RNA or DNA.[27]

The most widely used testes are the three generations of enzyme immunoassays (EIAs) that have increasing sensitivity.[27] The predictive values of these tests are greatest in high-risk populations but the false-positive rates can be as high as 50-60% in low-risk populations. False negative results can also occur because antibodies remain undetectable for as long as 1-3 months after clinical onset of the illness.[27] Anti-HCV antibody is not a protective antibody, does not confer immunity, and is usually present simultaneously with the virus. The other serologic test is the high sensitivity recombinant immunoblot assay (RIBA), which is used primarily to confirm a positive EIAs result in a low-risk population.[27]

1.2.8 Management of patients co-infected with Hepatitis C Virus and HIV

Hepatitis C virus (HCV) has become a significant contributor to morbidity and mortality in people infected with HIV since the introduction of highly active antiretroviral therapy (ART).[28] The presence of HIV clearly has a negative effect on the natural history of HCV, although there is some debate over whether HCV influences the natural history of HIV.[28] Taking into consideration the prevalence of co-infection and the accelerated liver damage from HCV, treatment of chronic HCV infection is necessary in patients co-infected with HIV.[28] There are few studies of pegylated interferon and ribavirin in co-infected populations, but it seems that the treatment is well tolerated, although it is possibly less effective in this group.[28] ART in the setting of HCV infection also requires some special consideration, as there is an increased incidence of hepatotoxicity.[28] Treatment of co-infected patients requires

close monitoring as current therapies are not ideal in terms of effectiveness, and toxicity may be severe.[28]

2.0 STATEMENT OF THE PROBLEM

A large proportion of hospitalized children at MNH are due to HIV/AIDS associated illnesses. According to the 2003-2004 report of the department of paediatrics at MNH a total of 566 deaths occurred, of which 19.6% were due to HIV/AIDS related illnesses.[29] The management of HIV-infected children at this hospital follows the national guidelines for treatment and care of HIV-infected children.[30] In brief, HIV serology is followed by enumeration CD4 T lymphocytes (for those found to be HIV-infected). With these results children are then referred to Pediatrics HIV Care and Treatment Center (CTC), where they are categorized into two groups; those eligible to start ART and those who are not, based on the WHO recommendations for ART in children.[30] According to these recommendations, children eligible for treatment include those presenting with WHO clinical stage 3 or 4 or those with WHO stage 2 with CD4 less than 20% in children less than 18 months of age or CD4 less than 15% in children more than 18 months of age. The drugs used include Zidovudine (AZT), Lamivudine (3TC), and Nevirapine (NVP) for children less than 3 years, for those more than 3 years Nevirapine may be replaced by Efaviranz (EFV) as first line, Didanosine (ddI), Stavudine (d4T), and lopinavir/ritonavir co-formulation as second line.

The problems seen in this set up include;

i). There is limited information regarding hepatitis B and C viral co-infections among the HIV-infected children. The study of hepatitis C virus infection among children at MNH [26] was beset by the use of latex agglutination technology which has been shown to perform poorly compared to other assays.[26] The latex agglutination

technique is liable to cross reactions,[31] which may have contributed, at least in part, to the lack of association between the occurrence of the virus and known risk factors e.g. blood transfusion.[26] Furthermore, since the publication of the HCV study by Kitundu et al.[26] Several people have questioned the reported HCV prevalence of 7%.

ii). The lack of information of viral co-infections has led to treatment of children being treated as if they have HIV infection alone. Although the regimens used for treatment of HIV children include lamivudine, which could cover HBV, it is now clear that this drug is not as useful as other options. Drug-resistant mutants occur in a proportion of patients.[32] HBV develops resistance to 3TC frequently in HBV/HIV co-infected people. On the other hand, combination of Tenofovir Disoproxil Fumarate (TDF) with lamivudine also appeared to be more effective in suppressing development of lamivudine resistance (reference). Furthermore, simultaneous administration of lamivudine and TDF in HBV/HIV co-infected patients has profound reduction in HBV DNA viral load than lamivudine alone or with lamivudine followed by lamivudine and TDF in combination.[33]

iii). Although children with hepatitis viruses and HIV co-infections are more prone to increased risk of hepatotoxicity, this may also be due to antiretroviral therapy. This distinction has not been fully investigated in our setting. It would be interesting to compare liver function tests of children on ART with those who have not started treatment, as well as making comparisons for children with HIV infection alone versus those with viral co-infections.

2.1 Rationale

This study aimed to provide an update data on seroprevalence of HBV and HCV among HIV infected children. It also aimed to provide baseline data on the association between these viruses. Knowing the magnitude of the problem and its associated risk factors will help clinicians in planning screening strategies and treatment protocols for HIV co-infected children.

3.0 OBJECTIVES OF THE STUDY

3.1 Broad objective

To determine the seroprevalence and risk factors for HBV and HCV among HIV infected children aged 18 months to 17 years attending the paediatric HIV Care and Treatment Center (CTC) at MNH, Dar-es-Salaam, Tanzania.

3.2 Specific objectives

3.2.1 To determine the seroprevalence of HBV and HCV, among the HIV infected children by age and sex.

3.2.2 To determine the risk factors associated with HBV and HCV among the HIV infected children.

3.2.3 To determine the association between level of immunosuppression with HBV and HCV in HIV infected children.

3.2.4 To determine the association between HBV, and HCV among the HIV infected children.

3.2.5 To compare levels of ALT in HIV-infected children on ART and those not on ART

3.2.6 To compare levels of ALT in HIV-infected children who are co-infected with hepatitis virus with those who are not co-infected

4.0 MATERIAL AND METHODS

4.1 Study design: Cross sectional hospital based descriptive study.

4.2 Study setting

The study was conducted at the Paediatrics HIV CTC in Muhimbili National Hospital between April 2006 and August 2006. This is the largest referral, consultant and University teaching hospital in Tanzania. Its catchments areas include three districts in Dar es Salaam. It also receives referrals from all over the country. The paediatrics CTC at MNH receives about 15-20 HIV infected children per day. Children who are on ARVs are followed up monthly while those who are not on ARVs are followed every 2 to 3 months.

4.3 Study Population

All HIV infected children who were attending the Paediatrics HIV CTC at MNH during the study period were eligible. However, inclusion was subject to obtaining an informed verbal consent from the parent/guardian who was accompanying the child. Children included in this study were aged between 18 months and 17 years. Ascent was obtained from children more than 10 years of age. The following information was given to ensure that parents/guardians have the information needed to make an informed choice: a complete description of the aims of the study, potential benefits and risks, if any. Study personnel provided any other requested additional information. All patients' information and test results were confidentially kept.

4.4 Sample size

Calculations of sample size were based on the prevalence of hepatitis C virus reported among children at MNH[26], critical value, type I error of 5% and power of the study of 80%.

The sample size was calculated from the following formula

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

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

N= Estimated sample size

E= Margin of error 4%

P= 7.1%, based on a study by Kitundu et al (26)

$N= (1.96)^2 \times 7.1\% (100-7.1\%) / (4\%)^2$

N= 158.5 N= 160. This is the minimum sample.

4.5 Sampling procedure

Children were enrolled consecutively until the sample size was reached. Enrollment was on working days from Monday to Friday between 9 am and 3pm.

4.6 Interviews

Parents/guardians were interviewed using a standard structure questionnaire (Appendix 1) to obtain information regarding demographics, age, sex, and duration of illness, past medical history such as history of blood transfusion, history of parenteral treatment, family and social history. Patient record files were used to obtain information regarding HIV status and CD4 count. Only recent CD4 values

(within three months) were recorded. Children were considered to be immunosuppresed according to the following immunological classification[1].

Table 1 Immunological Classification Based on Total CD4 and % CD4 count (ANNECA 2005)

Immunologic category	**Age of the Child**		
	< 12 months	**1-5 years**	**6-12 years**
	CD4/μL (%)	**CD4/μL (%)**	**CD4/μL (%)**
1. No evidence of suppression	≥1500 (≥25)	≥ 1000 (≥25)	≥ 500 (≥25)
2. Evidence of moderate suppression	750-1499 (15-24)	500-999 (15-24)	200-499 (15-25)
3. Severe suppression	< 750 (<15)	< 500 (<15)	< 200 (<15)

4.7 Physical examination

A thorough physical examination was done according to standard clinical methods[34] General examination, followed by systemic examination was done. Genera examination included looking skin and mucous membrane for the presence of jaundice especially the sclera and mucous under the tongue, palmar pallor, lymph node enlargement by palpation. Abdominal examination to elicit enlargement of the liver and spleen was done.

4.8 Laboratory investigations

Laboratory investigations done included HBsAg and HCV Abs; these were done at the Department of Microbiology and Immunology of the Muhimbili University of Health and Allied Sciences. ALT was done at the Specialized Paediatrics Laboratory of the Muhimbili National Hospital.

4.8.1 Specimen collection

About five milliliters of venous blood was taken from the anterior cubital fossa of each child using a sterile syringe and needle after a thorough cleaning of venopuncture site with a swab soaked in 70% ethyl alcohol. The blood was collected in a five ml red top vacutainers (BD, NJ, USA). The collected blood was kept at the room temperature till the end of the clinic, when it was taken to the laboratory for precessing. Sera were separated by centrifugation at 3000 revolutions per minute, thereafter were aliquoted into 2 ml cryotubes (Nalge Nunc International, IL, USA) and stored at -20^0C until the time for assay.

4.8.2 Detection of hepatitis C IgG antibodies

IgG antibodies to HCV were detected using an ELISA technique (EIAgen HCV Ab Kit) according to the instructions of the manufacturer (Adaltis Italia S.p.A). In brief, this involved inoculation of diluted sample on microwells coated with highly purified antigens which contain sequences from the core, NS3, NS4 and NS5 regions of HCV. Exactly 50µl assay diluent was dispensed into all controls/calibrator and sample wells. The microplate was then incubated for 45 min at +37°C, thereafter was

washed with an automatic washer by delivering and aspirating 350µl/well of diluted washing buffer. This was followed by the addition of 100µl enzyme conjugate into each well. The microplate was then incubated for 45 min at +37°C and thereafter washed with automatic washer. Thereafter, 100µl chromogen/substrate mixture were dispensed and then incubated at room temperature (18-24°C) for 15 min. Finally, 100µl of 0.3M sulphuric acid solution was added to all wells to stop enzymatic reaction. The addition of an acid turned the positive controls and positive samples from blue to yellow.

The color intensity of the solution in each well was measured at 450nm filter in spectrophotometer (Adaltis Italia S.p.A). According to the manufacturer the specificity and sensitivity of this assay is 99.5% and 100%, respectively.

4.8.3 Detection of hepatitis B surface antigen (HBsAg)

Detection of HBsAg was done using ELISA technique (EIAgen HBsAg Kit) according to the instructions of the manufacturer (Adaltis Italia S.p.A). Briefly, by using micropipettes 150µl of the negative control were dispensed in triplicate, 150µl of calibrator in duplicate and then 150µl of the positive control in single followed by 150µl of each of the samples. Exactly 100µl of diluted enzymatic conjugate were dispensed in all wells except the blank. The microplate was then incubated for 120 min at +37°C and thereafter washed with an automatic washer buffer in 4 cycles. Exactly 200µl chromogen/substrate was added into all the wells including the blank well, followed by incubation at room temperature (18-24°C) for 30 min. Finally 100µl of 0.3M sulphuric acid was added to all wells to stop enzymatic reaction.

The color intensity of the solution in each well was measured at 450nm filter in spectrophotometer. According to the manufacturer the sensitivity and specificity of this assay is 100% and >99.5%, respectively.

4.8.4 Alanine aminotransferase (ALT)

Catalytic activity of ALT (E C 2.6.1.2) was determined in serum using a COBAS MIRA instrument after it was calibrated. The principal of the assay is based on the kinetic UV test according to the recommendation of the International Federation of Clinical Chemistry (IFCC) start reagent method.

Principle: L-Alanine + 2-oxoglutarate $\xleftrightarrow{ALT}$ L-glutamate + pyruvate

Pyruvate + NADH + H^+ $\xleftrightarrow{LDH}$ L-Lactate + NAD^+

The rate of NADH oxidation directly related to the ALT activity and is measured photometrically. (Roche laboratory Systems/ HOSPOC)

Normal ALT: 1-19 years 5-45μ/l

4.9 Statistical analysis

Data were entered, cleaned, and analyzed using EPI INFO version 3.3.2 and SPSS version 10.0.[35] The seroprevalence of HCV and HBsAg were expressed in percentages for the entire study group and by age and sex. Chi-Square (χ^2) was used to determine the association between level of immunosuppression with HBV and HCV. A p-value of 0.05 or less was taken to indicate significant association. Fisher's exact test was used where the expected frequency was less than 5. Univariate and multivariate regression were used to determine the associations

between elevation of ALT, immunosuppression, ART and hepatitis co-infection. The associations were presented as odds ratio (OR) together with 95% confidence intervals (C1) and were considered to be significant if the corresponding 95% C1 does not include one.

4.10 Ethical issues

An informed consent was obtained from the parent/guardian prior to enrollment and assent to those more than 12 years was also obtained. The following information was given during parent/guardian education to ensure that they have the information needed to make an informed choice; a complete description of the aims of the study, infectious agents that were screened, potential benefits and risks, blood collection procedures and assurance of confidentiality of any information given as well as test results. Study personnel provided any other requested additional information to parent/guardian. Children with hepatitis co-infection were followed up at the clinic and cared and managed accordingly.

4.11 Ethical clearance:

Ethical and research clearance was obtained from the Higher Degree Research and Publication Committee of the Muhimbili University of Health and Allied Sciences in Dar es Salaam, Tanzania. Permission to conduct the study was sought from MNH authorities.

5.0 RESULTS

A total of 167 children were recruited during the study period (April to august 2005). Among them, 88(52.7%) were males and 79(47.3%) were females, with a male to female ratio of 1.1:1.

Most of the children (49.7%) were aged 6-10 years (Table 2). There were only 3.6% more than 15 years (Table 2).

Table 2: Age and sex distribution of the study group

VARIABLES	N	%
Age (yrs)		
<2	7	4.2
2-5	37	22.1
6-10	83	49.7
11-15	34	20.4
>15	6	3.6
Sex		
Male	88	52.7
Female	79	47.3

The overall prevalence of hepatitis viral co-infection among the HIV infected children was about 15 % (25/167) (Table 3). The prevalence of was significantly

higher among girls (21.5%) than boys (9.0%). There was no association between age and hepatitis viral co-infection. (Table 3)

Table 3: Prevalence of hepatitis viral co-infection by age and sex

	Total N (%)	Hepatitis co-infection N (%)
Sex		
Male	88	8(9.0)
Female	79	17(21.5)
Age (yrs)		
<2	7	2(28.6)
2-5	37	4(10.8)
6-10	83	12(14.5)
11-15	34	6(17.6)
>15	6	1(16.7)
Total	**167**	**25(14.9)**

Two children (1.2%) were co-infected with HBsAg and 23(13.8%) were co-infected with HCV (Table 3). The prevalence of HCV was significantly higher among girls (20.3%) than boys (8%) (P = 0.02) (Table 4). However there were no sex differences in the occurrence of HBsAg P=0.93 (Table 4). Agewise, the occurrence of HCV was highest (28.5%) in children aged less than two years and in children older than 10 years (Table 4). The same table shows that HBsAg were only seen in children aged

6-10 years. However the age differences were not statistically significant, with P-values for HBsAg and HCV being 0.72 and 0.69, respectively (Table 4).

TABLE 4: Seroprevalence of HBsAg and HCV co-infection by age and sex among HIV infected children

Sex	Total sample	HBsAg seropositive N (%)	HCV seropositive N (%)
Male	88	1(1.1)	7(8.0)
Female	79	1(1.3)	16(20.3)
		P=0.93	*P=0.02*
Age group (yrs)			
<2	7	0 (0)	2 (28.5)
2-5	37	0 (0)	4 (10.8)
6-10	83	2 (2.4)	10 (12.0)
11-15	34	0 (0)	6 (17.6)
>15	6	0 (0)	1 (16.7)
		P=0.72	*P=0.69*
Total	**167**	**2 (1.2)**	**23 (13.8)**

There were 148 (88.6%) children with history of injection, 26 (15.6%) had blood transfusion, 48 (28.7%) had uvulectomy and 2 (1.2%) were sexually abused. Among

those with history of injection 1(0.7%) had HBsAg co-infection and 19(12.8%) had HCV. However, there was no association between these risk factors and the occurrence of hepatitis viral co-infection (Table 5).

Table 5: Risk factors associated with HBsAg and HCV co-infection in HIV infected children.

Risk factor	Total sample	HBsAg N (%)	HCV N (%)
Injection			
Yes	148	1(7)	19(12.8)
No	19	1(5.3)	4(21.1)
		*P=0.21	*P=0.30
Blood transfusion			
Yes	26	0(0)	5(19.2)
No	141	2(1.4)	18(12.8)
		*P=1.00	P=0.36
Uvulectomy			
Yes	48	1 (2.1)	9 (18.8)
No	119	1 (0.8)	14 (11.8)
		*P=0.49	P=0.32
Sexual abuse			
Yes	2	0 (0)	0 (0)
No	165	2 (1.2)	23 (13.8)
	*P=1.00		*P=1.00

* Fisher's exact test

Among 167 HIV infected children 59 (35.3%) had no evidence of immunosuppression, 59 (35.3%) had moderate immunosuppression and 49 (29.3%) had severe immunosuppression (Table 5). There was no statistically significant association between immune status and hepatitis viral co-infection, *P=0.12* (Table 6)

Table 6: Prevalence of hepatitis viral co-infection by level immunosuppression

Level of immunosupression	**Total sample**	**Hepatitis B/C viral co-infection N (%)**	**No hepatitis N (%)**
No evidence of immunosuppression	59	5 (20.0)	54 (38.0)
Moderate immunosuppression	59	13 (52.0)	46 (32.4)
Severe immunosuppression	49	7 (28.0)	42 (29.6)
		***P=0.12*	
Total	**167**	**25**	**142**

** Chi-square

None of the investigated children had HBV and HCV dual infection (Table 7). The two children who were HBsAg positive were HCV negative. All the 23 HCV seropositive children were negative for HBsAg (Table 7). There was no association between HBsAg and HCV. P= 1.00 (Table 7).

Table 7: Association between HBsAg and HCV

	HCV		
HBsAg	Negative	Positive	**Total**
	N (%)	N (%)	
Negative	142 (86.1)	23 (13.9)	165
Positive	2 (100)	0 (0)	2
Total	144	23	167

Fisher's exact test

Among 167 children involved in the study 15(9%) had elevated ALT (Table 8). The prevalence of elevated ALT among children co-infected with hepatitis B or C virus was 20.0%, while that without hepatitis co-infection was 7.0%. There was a statistically significant association between hepatitis viral co-infection and elevated ALT. P=0.05 (Table 8).

Of the children studied, 125 (74.9%) were on ART. (Table 7 and among them 12(9.6%) had elevated ALT. The prevalence elevated ALT among children who

were not on ART 7.1%. There was no statistically significant association between elevated ALT and being on ART (P=0.76) (Table 8).

Of the 25 children who had co-infection with hepatitis, 22(88%) were on ART and among them 5(22.7%) had elevated ALT. None of the children not on ART had elevated ALT. However, there was no statistically significant association between elevated ALT and being on ART (Table 8).

Table 8: Association between elevated ALT by ART and hepatitis B and C viral co-infection

	ALT		Total
	Normal N (%)	**High N (%)**	**N**
Hepatitis B/C viral co-infection			
Yes	20 (80.0)	5 (20.0)	25
No	132 (92.9)	10 (7.0)	142
		**P= 0.05*	
ART			
Yes	113 (90.4)	12 (9.6)	125
No	39 (92.9)	3 (7.1)	42
		**P= 0.76*	
Hepatitis B/C viral co-infection & ART			
Yes	17 (77.3)	5 (22.7)	22
No	3 (100.0)	0 (0.0)	3
		**P= 1.00*	

Fisher's exact test

There is significant association between hepatitis co-infection and elevated ALT. children co-infected with hepatitis had 4 times more risk of elevated ALT than those without hepatitis. OR 3.99, 95% CI 1.12-14.19 (P=0.03) Table 9

Table 9: Association between elevated ALT and level immunosuppression, ART and hepatitis viral co-infection

	Univariate		Multivariate	
	OR	**95% CI**	**OR**	**95% CI**
Immunosuppression				
No evidence	1.0	-	1.0	-
Moderate	0.74	0.16-3.45 (P=0.69)	0.55	0.11-2.75 (P=0.47)
Severe	2.68	0.76-9.52 (P=0.13)	2.53	0.69-9.28 (P=0.16)
ART				
Not on ART	1.0	-	1.0	-
On ART	1.38	0.37-5.15 (P=0.63)	0.86	0.21-3.47 (P=0.83)
Hepatitis B/C viral co-infection				
No	1.0	-	1.0	-
Yes	3.29	1.02-10.65 (P=0.04)	3.99	1.12-14.19 (P=0.03)

6.0 DISCUSSION

The aim of this study was to determine the seroprevalence of HBV and HCV co-infection and to identify potential risk factors associated with these infections among HIV infected children aged 18 months to 17 years who were attending the paediatrics Care and Treatment Center at MNH in Dar-es-Salaam.

This study showed a high prevalence (15%) of hepatitis co-infection, which is approximately one out seven HIV-infected children. The prevalence of HCV alone observed in this study was 13.8%, which is significantly higher than 7.1%, that was reported by Kitundu *et al*[26] in post-transfused Tanzanian children. The difference between these studies could be due the nature of the studied populations. In the present study all children were HIV infected and were therefore more prone to opportunistic infection including hepatitis viruses. Secondly, the methods used were different, in this study antibodies to HCV was detected using an ELISA technique while in his study agglutination test was used which is relatively less sensitive and less specific due to potential cross reactions [51]. However, it is important that these ELISA based results are confirmed with more sensitive technique such as HCV-RNA by PCR, since it has been shown that some positive ELISA results becomes negative when confirmed by PCR.[52]

The seroprevalence of HBsAg alone in this study was 1.2%, which is significantly lower than 12% observed by Kitundu *et al*[53] among children transfused with anti-HIV negative donor blood at the same hospital (unpublished observation). The lower prevalence in this study is probably due to Hepatitis B vaccination program introduced in 2002 in our country. It has been shown that vaccination induces a rapid

decrease in the number of acute Hepatitis B infections and has a secondary effect of decrease in relative sequels.[54]

The prevalence of HBsAg observed in this study is comparable with those of Pellizer *et al*[56] who found overall prevalence of HBsAg was 4.4% among subjects aged one to 76 years living in urban and rural areas in Tanzania[56] and similar to a study conducted in Kenya by Rana *et al*[57] who found a prevalence of hepatitis B to be 4%, among African children infected with HIV.

In this study the prevalence of HCV/HBV was not associated with age (18 months to 17 years). One limitation of this study was that children below 18 months were not investigated due to methodological limitations associated with the use of IgG based ELISA.[52] This limitation may, at least in part, have obscured age trends.

Regarding HCV, females were more affected 17(21.5%) than males 8(9%) a finding that is consistent with a study done by Kitundu *et al*[53] in the same setting. The reason for the differences in sex is unclear, although others have speculated biological differences in susceptibility or response to infection.[55] This observation warrants further investigation.

In this study, 148 (88.6%) children had history of injection, 26 (15.6%) had Blood transfusion, 48 (28.7%) had uvulectomy and 2 (1.2%) were sexually abused. However none of these potential risk factors was associated with occurrence of the hepatitis viruses. There are other risk factors such as tradition-practitioner treatment of already ill children, with infected razors which could be the possibility of transmission but were not studied in the current study. The lack of association between either HBV or HCV with the investigated risk factors observed in this study

has also been observed in another study conducted in the same hospital by Kitundu *et al* [53]. This finding coupled with the young age of infection, may indicate that possibly these children acquired these viral infections vertically rather than horizontally. To establish the presence of vertical transmission, further studies that will investigate both the mothers and infants are recommended. It is known that women who are seropositive for both HBsAg and HBeAg vertical transmission is approximately 90% and that vertical transmission occurs in up to 10% of neonates when infection occurs in the first trimester and in 80 -90% of neonates when acute infection occurs in the third trimester [ACOG educational bulletin 1998]. On the basis of this fact the infants born to HBsAg and HBeAg positive mothers has to be tested for HBsAg and HBeAg at birth, 6months of age and at 18 months of age.[58] As diagnosis is done in lower age group detection of HBV-DNA by PCR should be preferred since some infants with ELISA- positive HBsAg in the first month of life may become negative with PCR.[59] To establish vertical transmission of HCV, infants of mothers with positive anti-HCV and HCV RNA by PCR, should be tested for HCV RNA by PCR in the first three days of life then at 6 weeks, every 3 months up to 12 months and every 6 months up to 24 months as proposed by other investigators.[60, 62]

This study showed no association between HBV and HCV, a finding that is in keeping with observations by Matee *et al*[51] and Wadell *et al*[61] among blood donors in the same Hospital. The lack of association in the occurrence of these two viruses could lend support the argument that their epidemiology is different in our setting [51, Due to this, and the fact that the management of the two viruses is different it is

important that HIV infected children presenting at the Paediatrics CTC at MNH are routinely screened for both viruses and are managed accordingly.

Notably, children with hepatitis co-infection had significantly lower levels of CD4 (Table 6). This is expected due to the influence of HCV/HBV infections on the natural history of HIV disease. It has been shown that infection with HCV increases in HIV viral load and CD4 cell counts declines as well as HIV disease progression even in patients receiving ART.[63] Indeed HCV RNA levels are inversely related to $CD4^+$ T-cell count ($P < 0.05$).[64]

About 8.9% of the investigated children had elevated ALT; a higher prevalence of raised ALT (20.0%) was among children co-infected with hepatitis virus compared with 7.0% without hepatitis co-infection (Table 8). This was statistically significant (OR 3.99, 95% CI 1.12-14.19). These finding is expected since hepatitis viruses are known to elevate liver enzyme.[65] It was observed that 9.6% of children on ART had raised ALT, compared with 7.1% of children who were not on ART, implying that there was no significant association between ART and raised ALT in the investigated group. This is contrary to findings of a number of studies that have shown association between ART usage and elevated ALT.[66, 67, 68] Children recruited in this study were on first line regimen, which does not contain protease inhibitors, which is associated with potential risk of elevated ALT than other antiretrovirals.[68] There has also been reports that different genotype of hepatitis C virus differ in their ability to cause hepatotoxicity, with genotype 3 being more hepatotoxic than genotypes 1, 2,

and 4.[67] It would be interesting to perform genotyping of circulating HCV strains to better understand association with liver pathology.

The high prevalence of hepatitis co-infection among HIV infected children indicates the need to revisit the current guidelines for the clinical management of HIV and AIDS. The regimen (zidovudine, lamivudine, and nevirapine for children < 3 years, with efaviranz replacing nevirapine for children > 3 years and didanosine, abacavir and ritonavir boosted lopinavir as second line) does not take care for the hepatitis co-infection. Although lamivudine works for both HBV and HIV, prolonged lamivudine therapy can result in drug-resistant HBV mutants and has also been associated with hepatitis flares.[69,71] The combination of lamivudine and tenofovir (which is an acyclic nucleotide reverse transcriptase inhibitor) could be considered in the treatment of children with proven HBV/HIV co-infection.[69,73]

Finally, the higher prevalence of HCV (13.8%) observed in this study calls for introduction of confirmation tests such as HCV-RNA by PCR at HIV care and treatment center in MNH. Children who will be subsequently proven to be infected with HCV should be monitored closely and provided with, preferably peginterferon and ribavirin combination therapy, which is a standard treatment of HCV/HIV co-infection.[74]

7.0 CONCLUSIONS

1. There is high seroprevalence (15%) of hepatitis co-infection among HIV infected children attending the peadiatrics HIV care and treatment center at the Muhimbili National Hospital. However, there was no association in the occurrence of HBV and HCV.

2. The occurrence of hepatitis B and C viruses was not associated with any of the investigated risk factors (i.e. history of injection, blood transfusion, uvulectomy and sexual abuse).

3. Children with hepatitis viral co-infection had significantly higher levels of ALT and lower counts of CD4+ lymphocytes.

8.0 RECOMMENDATIONS

1. Hepatitis B and C viruses should be routinely screened in HIV infected children presenting at the HIV care and treatment centre at MNH.

2. The laboratory that serves for the HIV CTC at MNH should be provided with reagents for HCV RNA and HBV DNA PCR that will facilitate diagnosis of these viruses at younger age (< 18 months).

3. Further study is needed to determine genotype of HBV and HCV so as to better understand the epidemiology of these viruses and as well as provide essential information for vaccine development.

4. There is a need of assessing vertical transmission of HCV by HCV RNA PCR in infants borne HCV seropositive mothers.

9.0 STUDY LIMITATIONS

1. Being a hospital based study the observed prevalence does not reflect the true prevalence of HBV and HCV in the population.
2. The information about risk factors was based on the memory of parents/guardians, which is subject to recall bias.
3. Detection of HCV was based on finding IgG antibody against the virus in blood, while a more realistic test would have detection of HCV RNA.
4. The duration of ART was not documented, thus limiting information regarding the association between ART and ALT elevation.

10.0 REFERENCES

1. UNAIDS-United Nations Joint Programme on HIV/AIDS December 2006 Report on the Global AIDS Epidemic *http://data.unaids.org/pub/EpiReport/2006/2006_EpiUpdate_en.pdf*
2. African Network for care of Children affected by AIDS (ANECCA), 2005, A handbook on Paediatric AIDS in Africa. http://www.fhi.org/en/HIVAIDS/pub/guide/mansl.htm
3. Kawo G, Karlson K, Lyamuya E, *et al.* Prevalence of HIV type 1 infection associated clinical feature and mortality among hospitalized children in Dar es Salaam Tanzania. *Scand J Infect Dis* 2000;**32**:357-363.
4. Massawe H, Tumwesigye N, Kisenge R, Kiwanuka J, Kanave E, Massawe A, *et al.* Comprehensive paediatric HIV/AIDS care course for health workers by ANECCA. Dar-es-salaam, Tanzania. 21-25 November 2006, Kurasini Training center.
5. Raymond T Chung, Arthur Y kim and Bruce Polsiky HIV/Hepatitis B and C co- infection: pathogenic interactions, natural history and therapy. *J HIV Ther* 2003;**8**:77-84.
6. Bruno R, Sacchi P, Puoti M, Soriano V, Filice G. HCV chronic hepatitis in patients with HIV: clinical management issues. *AIDS* 2000;22:**14**:2895-2902.
7. Lee W, Dieterich D. Challenges in the management of HIV and hepatitis C virus co-infection. *J Viral Hepat* 2004;11:**97**-107.

8. Chung RT, Kim AY, Polsky B. HIV/Hepatitis B and C co-infection: pathogenic interactions, natural history and therapy. *J HIV Ther* 2003;**8**:77-84

9. Konopnicki D. Hepatitis B and HIV: prevalence, AIDS progression, response to highly active antiretroviral therapy and increased mortality in the EuroSIDA cohort. *AIDS* 2005;**19**:593-601.

10. Horvath J, Raffanti SP. Clinical aspects of the interactions between human immunodeficiency virus and the hepatotropic viruses. *Clin Infect Dis* 1994;**18**:339-347.

11. Gatanaga H, Yasuoka A, Kikuchi Y, Tachikawa N, Oka S. Influence of prior HIV-1 infection on the development of chronic hepatitis B infection. *Eur J Clin Microbiol Infect Dis* 2000;**19**:237-239.

12. Weller IV, Brown A, Morgan B, Hawkins A, Briggs M, Waite J, *et al.* Spontaneous loss of HBeAg and the prevalence of HTLV-III/LAV infection in a cohort of homosexual hepatitis B virus carriers and the implications for antiviral therapy. *J Hepatol* 1986;**2**:9-16.

13. Perrillo RP, Regenstein FG, Roodman ST. Chronic hepatitis B in asymptomatic homosexual men with antibody to the human immunodeficiency virus. *Ann Intern Med* 1986;105:382-383.

14. Krogsgaard K, Lindhardt BO, Nielson JO, Andersson P, Kryger P, Aldershvile J, *et al.* The influence of HTLV-III infection on the natural history of hepatitis B virus infection in male homosexual HBsAg carriers. *Hepatol* 1987;7:37-41.

15. Waite J, Gilson RJ, Weller IV, Lacey CJ, Hambling MH, Hawkins A, *et al.* Hepatitis B virus reactivation or reinfection associated with HIV-1 infection. *AIDS* 1988;**2**:443-448.

16. Mastroianni CM, Trinchieri V, Santopadre P, Lichtner M, Forcina G, D'Agostino C, *et al.* Acute clinical hepatitis in an HIV-seropositive hepatitis B carrier receiving protease inhibitor therapy. *AIDS* 1998;**12**:1939-1940.

17. Rodriguez-Mendez ML, Gonzalez-Quintela A, Aguilera A, Barrio E. Prevalence, patterns, and course of past hepatitis B virus infection in intravenous drug users with HIV-1 infection. *Am J Gastroenterol* 2000;**95**:1316-1322.

18. Gilson RJ, Hawkins AE, Beecham MR, Ross E, Waite J, Briggs M, *et al.* Interactions between HIV and hepatitis B virus in homosexual men: effects on the natural history of infection. *AIDS* 1997;**11**:597-606.

19. Kibassa CG, Mwakagile DSM, Kitundu JA, Msengi AE Determination of Vertical Transmission Rate of Hepatitis B virus Infection in Dar-es-Salaam, Tanzania.*Tanz Med J* 2004;**19**:21-24.

20. Howard J. Warman, M.D. Hepatitis B.Cpmcnet.columbia.edu/dept/gi/hepB.html-14k

21. Nunez M and Soriano V. Management of patients co-infected with hepatitis B virus and HIV. *Lancet* 2005;**5**:374-382.

22. HIV and Hepatitis Virus Co-infection: The Center of Two Epidemics http://www.retroconference.org/2001 (February 2006).

23. Opportunistic Infections (OIs) *www.aidsmeds.com/lessons/StartHere8.htm - 22k aids.about.com/od/hepatitisbasics/a/hivhepc.htm - 28k* (February 2006).

24. Resti M.; Azzari C.; Bortolotti F. Hepatitis C Virus Infection in Children Coinfected with HIV: Epidemiology and Management. *Paediatr Drugs* 2002;**4**:571-580.

25. Kebudi R, Ayan I, Yilmaz G, Akici F, Gorgun O, Badur S. Seroprevalence of hepatitis B, hepatitis C, and human immunodeficiency virus infections in children with cancer at diagnosis and following therapy in Turkey. *Med Ped Onc* 2000;**32**:102-105.

26. Kitundu J, Msengi A, Matee M, Fataki M, Kazimoto T, Mpembeni R, *et al.* Post-transfusion hepatitis C seroprevalence in Tanzanian children. *Ann Trop Paediatr* 2001; **21**:343-348.

27. Behrman RE, Nelson Textbook of paediatrics, John D Viral Hepatitis.Thomson press India: Elsevier, 2004;17th Ed:1324-1332.

28. Lee W, Dieterich D. Challenges in the management of HIV and hepatitis C virus co-infection. *J Viral Hepat* 2004;**11**:97-107.

29. Kazimoto T, Mwamteme H, Massawe H, Massawe A. Annual departmental rreport of morbidity and mortality. Muhimbili National Hospital, Department of Paediatrics and Child Health - 2003-2004 report.

30. Mugusi F, Bakari M, Massawe A, Mbwambo J, Materu M, Macharia E, National guidelines for clinical management of HIV and AIDS 2nd Ed 2005:107-115.

32. Dore GJ, Cooper DA, Barrett C, *et al.* Dual efficacy of lamivudine treatment in human immunodeficiency virus/hepatitis B virus co-infected persons in a randomized, controlled study (CAESAR). *J Infect Dis* 1999;**180**:607-613.

33. Jain MK, Comanor L, White C, Kipnis P, Elkin C, Leung K, *et al.* Hepatitis B virus (HBV) response to therapy with lamivudine (LAM) and tenofovir (TFV) in HBV/HIV co-infected patients: impact of genotype and treatment regimen. In: Program and abstracts of the 55th Annual Meeting of the *American Association for the Study of Liver Diseases;* October 29-November 2, 2004;Boston. Abstr 1168.

34. Hatchison R. The examination of Children. In Hatchison's clinical methods. 20th Ed. Edited by Swash M. London: Royal Hospital;1995;:365-385.

35. SPSS Inc version 10.0. *The statistical Package for the Social Sciences.* Chicago, II: SPSS Inc, 2004.

36. Atina JO, Ogutu EO, Hardison WG and Mumo J Prevalence of Hepatitis A,B,C and HIV seropositivity among patients with acute icteric Hepatitis at the Kenyatta National Hospital Nairobi *East Afr Med J* 2004;**81**:183-187.

37. Eskild A, Magnus P, Petersen G, Sohlberg C, Jensen F, Kittelsen P, *et al.* Hepatitis B antibodies in HIV-infected homosexual men are associated with more rapid progression to AIDS. *AIDS* 1992;**6**:571-574.

38. O'Connor JB, Imperiale TF, Singer ME. Cost-effectiveness analysis of hepatitis A vaccination strategies for adults. *Hepatol* 1999;**30**:1077-1081.

39. Diamondsstone LS, Blakley SA, Rice JC, Clark RA, Goedert JJ. Prognostic factors for all-cause mortality among hemophiliacs infected with human immunodeficiency virus. *Am J Epidemiol* 1995;**142**:304-313.

40. Twu SJ, Detels R, Nelson K, Visscher BR, Kaslow R, Palenicek J, *et al* Relationship of hepatitis B virus infection to human immunodeficiency virus type 1 infection. *J Infect Dis* 1993;**167**:299-304.

41. Solomon RE, VanRaden M, Kaslow RA, Lyter D, Visscher B, Farzadegan H, *et al*. Association of hepatitis B surface antigen and core antibody with acquisition and manifestations of human immunodeficiency virus type 1 (HIV-1) infection. *Am J Public Health* 1990;**80**:1475-1478.

42. Zylberberg H, Pol S. Reciprocal interactions between human immunodeficiency virus and hepatitis C virus infections. *Clin Infect Dis* 1996;**23**:1117-11125.

43. Garcia-Samaniego J, Soriano V, Castilla J, Bravo R, Moreno A, Carbo J, *et al*. Influence of hepatitis C virus genotypes and HIV infection on histological severity of chronic hepatitis C. The Hepatitis/HIV Spanish Study Group. *Am J Gastroenterol* 1997; **92**:1130-1134.

44. Benhamou Y, Bochet M, Di Martino V, Charlotte F, Azria F, Coutellier A, *et al*. Liver fibrosis progression in human immunodeficiency virus and hepatitis

C virus coinfected patients. The Multivirc Group. *Hepatol* 1999;**30**:1054-1058.

45. Soto B, Sanchez-Quijano A, Rodrigo L, del Olmo JA, Garcia-Bengoechea M, Hernandez-Quero J, Rey C, *et al.* Human immunodeficiency virus infection modifies the natural history of chronic parenterally-acquired hepatitis C with an unusually rapid progression to cirrhosis. *J Hepatol* 1997;**26**:1-5.

46. Sanchez-Quijano A, Andreu J, Gavilan F, Luque F, Abad MA, Soto B, *et al.* Influence of human immunodeficiency virus type 1 infection on the natural course of chronic parenterally acquired hepatitis C. *Eur J Clin Microbiol Infect Dis* 1995;**14**:949-953.

47. Darby SC, Ewart DW, Giangrande PL, Spooner RJ, Rizza CR, *et al.* Mortality from liver cancer and liver disease in haemophilic men and boys in UK given blood products contaminated with hepatitis C. UK Haemophilia Centre Directors' Organisation. *Lancet* 1997;**350**:1425-1431.

48. Di Martino V, Esteban JI, Tainturier M-H, Benhamou Y, Bochet M, Opolon P, *et al.* Impact of HIV co-infection on the long-term follow-up of HCV-related cirrhosis. *Hepatology*, 2000;**32**:p.A203.

49. Macias J, Pineda JA, Leal M, Abad MA, Garcia-Pesquera F, Delgado J, *et al.* Influence of hepatitis C virus infection on the mortality of antiretroviral-treated patients with HIV disease. *Eur J Clin Microbiol Infect Dis* 1998;**17**:167-167.

50. Wright TL, Hollander H, Pu X, Held MJ, Lipson P, Quan S, *et al.* Hepatitis C in HIV-infected patients with and without AIDS: prevalence and relationship to patient survival. *Hepatol* 1994;**20**:1152-1155.

51. Matee M, Magesa P, Lyamuya E. Seroprevalence of Human immunodeficiency virus, hepatitis B and C and syphilis infections among blood donors at Muhimbli National Hospital in Dar Es Salaam, Tanzania. *BMC Public Health* 2006;doi: 10.1186/1471-2458-6-21.

52. Robert J., Betty A., Ortiz-Conde, Rachel K., Paul MB, Cheng-DW, *et al.* Hepatitis C Virus Genotype 4 in Ugandan Children and Their Mothers. http://www.cdc.gov/ncidod/EID/vol12no09/04-1068.htm (April 2007).

53. Kitundu J, Matee MNI, Msengi AE, Maulidi F, Kazimoto T, Munubhi EK, *et al.* Seroprevalence of Hepatitis C virus and Hepatitis B infection in Tanzanian children transfused with Anti-HIV negative donor blood aged between 15 months and 5 years. (Unpublished observation).

54. Ranger-Rogez S, Denis F. Hepatitis B mother-to-child transmission. *Expert Review of Anti-infective Therapy*, 2004;**2**:133-145.

55. Ronald B. Risk factors for Perinatal Transmission of HCV and Higher Incidence of Infection in male than in female infants. *J Infect Dis* 2005;**192**:1880-1889.

56. Pellizer G, Ble C, Zamperetti N, Stroffolini T, Upunda G, Rapicetta M *et al.* Serological survey of hepatitis B infection in Tanzania. *Public Health* 1994;**108**:427-431.

57. Rana C, Gareth R, Dimitra B, Alexandra M, Jedediah R, Angelo D et al. Viral co-infection among Children Infected with HIV Type 1. *Clin Infect Dis* 2003;**36**:922-924

58. Biswas SC, Gupta I, Ganguly NK, Chawla Y, Dilawari JB. Prevalence of hepatitis surface antigen in pregnant mothers and its perinatal transmission. *Trans R Soc Trop Med Hyg* 1989;**83**:698-700.

59. Ferrero S, Lungaro P, Bruzzone B, Gotta C, Bentivoglii G, Ragni N. Prospective study of mother-to-infant transmission of hepatitis C virus: a 10-year survey (1990-2000). *Acta Obstet Gynecol Scand* 2300;**82**:229-234.

60. Peixoto MF, Matto AA, Remiao JOR, Alexandre COP, Lemos SK, Azevedo AMP. Vertical transmission of hepatitis C virus in a hospital in southern *Brazil Arq Gastroenterol* 2004;**41**:84-87.

61. Waddell RD, Magesa PM, Pallangyo KJ, Matee M, Bakari M, von Reyn F, Ziermann R. Co-infection with HIV and HCV in a Blood Bank Population in Dar es Salaam, Tanzania. *J Clin Virol* 2006 May 22; [Epub ahead of print] PMID: 16723273 [PubMed - as supplied by publisher]

62. Mok J, Pembrey L, Tovo P-A, Newell M-L. When does mother to child transmission of hepatitis C virus occur? *Arch Dis Fetal Neonatal Ed* 2005;**90**:F156-F160. doi: 10.1136/adc.2004.059436.

63. Bonacini M, Govindarajan S, Blatt LM, Schmid P, Conrad A, Lindsay KL. Patients co-infected with human immunodeficiency virus and hepatitis C virus demonstrate higher levels of hepatic HCV RNA. *J Viral Hepat* 1999;**6**:203-208.

64. Janice M, Gloria C, Sharon D, Regina W, Angela C, Richard M, *et al.* Comparison of Hepatitis C Viral Loads in Patients with or without Human Immunodeficiency Virus. *Clin Vac Immunol* 2001;**8**:690-694.

65. Brinker M, Wit FW, Wertheim-van PM, Jurriaans S, Weel J, Leeuwen R, *et al.* Hepatitis B and C virus co-infection and the risk for hepatotoxicity of highly active antiretroviral therapy in HIV-1 infection. *AIDS.* 2000;**22**:2895-2902.

66. Alfredo A, Nathan C, Simon C, Wolfram G, Jens L, Giorgio P, *et al.* Short statement of the first European consensus conference on the treatment of chronic Hepatitis b and c in HIV co-infected patients. *J Hepatol* 2005;**42**:615–624.

67. Torti C, Giuseppe L, Salvatore C, Massimo P, Mark N, Eugenia Q, *et al.* Incidence and risk factors for liver enzyme elevation during highly active antiretroviral therapy in HIV-HCV co-infected patients: results from the

Italian EPOKA-MASTER Cohort *BMC Infect Dis* 2005,**5**:58 doi: 10.1186/1471-2334-5-58

68. Sulkowski MS: Drug-induced liver injury associated with antiretroviral therapy that includes HIV-1 protease inhibitors. *Clin Infect Dis* 2004;**38**:90–97.

69. Juergen K Rockstroh Management of HIV and Hepatitis Virus Coinfections: *New Clinical Data.* 45th *ICAAC* 2005.

70. Bessesen M, Ives D, Condreay L, Lawrence S, Sherman KE. Chronic active hepatitis B exacerbations in human immunodeficiency virus-infected patients following development of resistance to or withdrawal of lamivudine. *Clin Infect Dis* 1999;**28**:1032-1035.

71. Wolters LM, Niesters HG, de Man RA, Schalm SW. Antiviral treatment for human immunodeficiency virus patients co-infected with hepatitis B virus: combined effect for both infections, an obtainable goal? *Antiviral Res* 1999;**42**:71-76.

72. Kasper D.L et al, Harrison's Principles of Internal medicine, The Mc Graw-Hill vol II 16th Ed 2005.

73. Cooper D, Dore G, Pozniak AL, DeJesus E, Tran S, Sayre J, Lu B, Westland C, Miller MD, Coakley DF, Cheng A. Tenofovir disoproxil fumarate and lamivudine combination therapy compared to lamivudine alone for HBV in therapy-naive HIV/HBV co-infected patients: 48-week interim results. In: Program and abstracts of the 10th Conference on Retroviruses and Opportunistic Infections; February 10-14,2003; Boston. Abstr no. 825.

74. Torriani FJ, Rodriguez-Torres M, Rockstroh JK, Lissen E, Gonzalez-Garcia J, Lazzarin A, Carosi G, Sasadeusz J, Katlama C, Montaner J, Sette H, Jr., Passe S, De Pamphilis J, Duff F, Schrenk UM, Dieterich DT. Peginterferon Alfa-2a plus ribavirin for chronic hepatitis C virus infection in HIV-infected patients. *N Engl J Med* 2004; **351**:438-450.