Impact of highly active antiretroviral therapy on liver function of under-five HIV-positive children in Southern Nigeria

Olugbenga M. Ajulo[1], Kayode M. Omole[2], Olanrewaju J. Moody[3], Ofonime T. Dixon-Umo[4] and Lekan O. Salami[5]

[1]Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmacy, University of Uyo, Uyo. Akwa-Ibom State. Nigeria.

[2]Department of Clinical Pharmacy and Pharmacy Administration, Faculty of Pharmacy, University of Ibadan, Ibadan. Oyo state. Nigeria.

[3]Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Ibadan. Oyo State. Nigeria.

[4]Department of Pediatrics, Faculty of Clinical Sciences, College of Medicine, University of Uyo Teaching Hospital,Uyo. Akwa-Ibom State. Nigeria.

[5]Pediatric PEPFAR clinic, University College Hospital, Ibadan. Oyo state. Nigeria.

Corresponding author: Olugbenga Ajulo
E-mail: ajugbeng@gmail.com Phone: +2347030262468

ABSTRACT

Background: Hepatotoxicity deserves serious attention due to mortality, morbidity and treatment discontinuation in HIV-seropositive patients.

Objectives: The study aimed at evaluating the impacts of Highly Active Antiretroviral Therapy (HAART) on liver function of under-five children in Southern Nigeria.

Method: In five hospitals, 238 under-five children were enrolled after ethical permission was received from Ethics and Research committees and written consent were obtained from participants' care-givers. Participants were divided into six groups: the HIV-seropositive either on HAART (group A, n= 91) or co-trimoxazole (group B, n= 11) and four other groups who were HIV-seronegative. Among this second cohort were those commenced on nevirapine for six weeks post-exposure (group C1, n= 24) and co-trimoxazole at 6 months (group C2, n= 18) or 18 months (group C3, n= 48) post-exposure. The last group received no medication (group D, n= 46). Initially, a blood sample of 2ml was obtained from each participant and was assayed for alanine aminotransferase (ALT) and aspartate aminotransferase (AST). After three- and six-months from the time of initial study, only group A participants were restudied for the liver enzymes' assay. Body mass index of the participants were also determined. Mean and standard deviation of the parameters of participants in group A was compared with those from other groups by using ANOVA. The differences in the parameters were considered significant at $p \leq 0.05$.

Results: Alanine aminotransferase (ALT) was significantly higher in group A compared to group B (12.8±11.0 IU/L vs 6.5±2.6 IU/L, p= 0.245), group C1 (12.8±11.0 IU/L vs 10.9±7.8 IU/L, p= 0.910), group C2 (12.8±11.0 IU/L vs 11.7±20.7 IU/L, p= 0.995), group C3 (12.8±11.0 IU/L vs 11.2±6.9 IU/L, p= 0.868), and group D (12.8±11.0 IU/L vs 5.8±3.4 IU/L, p= 0.001). After three and six months of monitoring, ALT of group A was significantly decreased by 39.3% (p= 0.001) and 50.6% (p= 0.000) respectively.

Conclusion: The elevated ALT of under-five HIV-infected children on HAART lowered after six months of monitoring.

Key words: Under-five HIV children, Alanine aminotransferase, Aspartate aminotransferase, Highly Active Antiretroviral Therapy.

Impact du traitement antirétroviral hautement actif sur la fonction hépatique des enfants séropositifs de moins de cinq ans au sud du Nigeria

Auteur correspondant: Olugbenga Ajulo
E-mail: ajugbeng@gmail.com Téléphone: +2347030262468

RESUME

Contexte: L'hépato-toxicité mérite une attention sérieuse en raison de la mortalité, la morbidité et l'arrêt du traitement chez les patients séropositifs au VIH.

Objectifs: L'étude visait à évaluer les effets de la thérapie antirétrovirale hautement active (ARVHA = HAART en anglais) sur la fonction hépatique des enfants de moins de cinq ans au sud du Nigeria.

Méthode: Dans cinq hôpitaux, 238 enfants de moins de cinq ans étaient inscrits après l'obtention d'une autorisation éthique de la part des comités d'éthique et de recherche, ainsi que l'obtention d'un consentement écrit auprès des personnes soignant les participants. Les participants étaient divisés en six groupes: les séropositifs soit sur HAART (groupe A, n= 91) ou cotrimoxazole (groupe B, n= 11) et quatre autres groupes qui étaient séronégatifs. Dans cette deuxième cohorte se trouvaient ceux qui ont commencé la névirapine pendant six semaines de post-exposition (groupe C1, n = 24) et cotrimoxazole à 6 mois (groupe C2, n = 18) ou 18 mois (groupe C3, n= 48) après-exposition. Le dernier groupe n'a reçu aucun médicament (groupe D, n= 46). Dans un premier temps, un échantillon de sang de 2 ml a été obtenu de chaque participant et a été testé pour l'alanine amino-transférase (ALT) et d'aspartate amino-transférase (AST). Après trois et six mois à partir du moment de l'étude initiale, seuls les participants du groupe A ont été réétudiés pour le dosage des enzymes hépatiques. Les indices de masse corporelle des participants ont également été déterminés. La moyenne et l'écart-type des paramètres des participants du groupe A ont été comparés à ceux d'autres groupes en utilisant ANOVA. Les différences dans les paramètres ont été considérés comme significatives à $p \leq 0,05$.

Résultats: L'alanine aminotransférase (ALT) était significativement plus élevée dans le groupe A par rapport au groupe B (12,8 ± 11,0 UI/L vs 6,5 ± 2,6 UI/L, p= 0,245), le groupe C1 (12,8 ± 11,0 UI/L vs 10,9±7,8 UI/L, p= 0,910), le groupe C2 (12,8 ± 11,0 UI/L vs 11,7±20,7 UI/L, p= 0,995), le groupe C3 (12,8±11,0 UI/L vs 11,2±6,9 UI/L, p= 0,868) et le groupe D (12,8±11,0 UI/L vs 5,8±3,4 UI/L, p= 0,001). Après trois et six mois de suivi, L'ALT du groupe A était significativement réduit de 39,3% (p= 0,001) et 50,6% (p= 0,000), respectivement.

Conclusion: L'ALT élevé des enfants de moins de cinq ans infectés par le VIH sous HAART a baissé après six mois de suivi.

Mots clés: Les enfants de moins de cinq ans infectés par le VIH, alanine aminotransférase, aspartate aminotransférase, thérapie antirétrovirale hautement active.

INTRODUCTION

There were 570,000 Acquired Immunodeficiency Syndrome (AIDS) related deaths among children aged 15 years and below. Human Immunodeficiency Virus (HIV) infection progresses rapidly in children and AIDS-related mortality among infants are exceptionally high.[1] Approximately 33% of untreated HIV- infected infants in the developing world die during the first year of life and greater than 50% die by the age of two years.[1] Sub-Saharan Africa is the worst-affected region. Of the global population, an estimated 22.5 million are people living with HIV, 1.3 million have died of HIV-related problems, and 1.8 million children are newly infected.[2]

Highly active antiretroviral therapy (HAART) decreases the viral load in HIV-infected individuals thereby increasing the CD4 cell count, consequently resulting in slowing the disease progression. This is followed by decreased mortality and improved quality of life.[3] Issues concerning severe adverse events are becoming increasingly evident, however, limiting the benefits of treatment in most patients.[4] Hepatic drug toxicity is exhibited by all classes of antiretroviral drugs which is indicated by rising liver enzymes such alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and sometimes by hypersensitivity reactions and steatohepatitis.[5]

The use of HAART as a frontline treatment of HIV is widely associated with drug-induced hepatotoxicity. This is responsible for frequent hospital visits and admissions with huge financial impacts.[6] Hepatotoxicity prevents continuous HIV-suppression over a long period of time.[6] Mortality due to discontinuation of HAART as a result of hepatotoxicity was reported to increase from 6% in 1996 to 31.8% in 1999.[7,8]

World Health Organisation (WHO) has commissioned systematic reviews on antiretroviral drug toxicities and laboratory monitoring strategies. The reviews highlighted some obvious and important gaps in the potential increase in the risk of toxicity that may result from long-term use of antiretroviral drugs, especially during pregnancy, breastfeeding and in children and adolescents.[9] It was concluded that regular and adequate monitoring of the toxicity should be incorporated into the routine laboratory studies. It is therefore important to monitor the use of antiretroviral drugs in developing countries with low income where drug toxicity may mimic other environmental or behavioural induced health problems. Surveillance of antiretroviral drug toxicity will lead to a better understanding of the long-term risk of the toxicity of antiretroviral therapy and optimize the management and prevention of the infection in the general population. Increased risk of toxicity that is associated with long term use of antiretroviral drugs, as well as renal and bone toxicities have necessitated further research most especially in resource limited countries of the world.[9]

Mechanisms of drug-induced hepatotoxicity include direct toxicity, hypersensitivity reactions, mitochondrial toxicity and metabolic abnormalities.[10] Drugs which are metabolized in the liver by the cytochrome P450 enzymes may cause hepatotoxicity. Idiosyncratic polymorphisms of enzymatic complexes may lead to a significant heterogeneity in drug metabolism leading to hepatotoxicity in susceptible individuals. Anti-oxidation stress mechanism predicts spontaneous normalization in the levels of alanine aminotransferase and aspartate aminotransferase as HAART intake continues.[11] Hypersensitivity reactions are idiosyncratic reactions of the host and are not related to the dose of the drug. Hepatotoxicity due to mitochondrial toxicity may lead to liver failure. In some individuals on HAART, insulin-resistance and non-alcoholic steatohepatitis may lead to development of hepatotoxicity.[8]

METHOD

Study setting:

The study participants were patients in the following hospitals; Federal Medical Center, Umuahia, University of Uyo Teaching Hospital, University College Hospital, Ibadan, Emmanuel Hospital, Eket and St Luke Hospital, Anua.

Study design:

This was a prospective observational study.

Sampling method and sample size determination:

The prevalence of HIV among 0-5 years old children in Nigeria is unknown, hence, an assumed prevalence of 50% was used. Raosoft software package was used to determine a sample size of 218 at 5% error margin and 95% confidence interval with a provision for 10% drop out.[12]

Two hundred and thirty-eight (238) under-five children were enrolled from five hospitals for the study. The participants were divided into six groups: the HIV-seropositive on either HAART (group A, n= 91) or co-trimoxazole (group B, n= 11) and four other groups who were HIV-seronegative. Among these other groups were those commenced on nevirapine for six weeks post-exposure after birth (group C1, n=24) and co-trimoxazole for six months (group C2, n= 18) or 18 months (group C3, n= 48) post-exposure. The last group were seronegative children from seronegative mothers who received no medication (group D, n= 46). All the

participants were breastfed exclusively for 6 months except those in group D who were breastfed for 12 months. The involvement of group D participants in this study was very important in order to fill the gap made in other study where control arm consisted of HIV-exposed children on previous mono antiretroviral therapy. The children in that study were also exposed to the HAART regimen received by their mothers through breastfeeding which could also influenced results of the study.[13] However, we decided to make a difference in our study by using children who were not exposed to either HIV-infection or HAART regimens as our control arm. US Federal regulations allow research with children that involves greater than minimal risk and no prospect of direct benefit when such research presents an opportunity to know, avoid and reduce a serious problem affecting the health or welfare of children.[14]

Drug development is presently focused on adults while less than 25% of drugs in the US and European Markets are indicated for use in paediatrics.[15] The United States Food and Drug Administration made regulations and Congress passed laws to ensure that marketed drugs would be evaluated for safety and efficacy in infants and children.[16] Ethical considerations are central to research design and practice. However, in attempting to balance conflicting values of advancing scientific knowledge and protecting human volunteers, it occasionally poses challenges. The choice of control arm is an aspect of research design involving entwined ethical and scientific issues.[17]

Ethical consideration:

Ethical permission was granted by Ethics and Research Committees at five hospitals to commence the study. Written consent was obtained from the care-givers of the children who participated in the study.

Inclusion criteria:

Under-five asymptomatic HIV-infected children participated in the three phases of the study.

Exclusion criteria:

Children beyond 5 years at the start of the study and asymptomatic HIV-infected children were not allowed to participate in the study. HIV-exposed children and seronegative-children from seronegative-mother were not allowed to participate in the second and third phases of the study.

Data collection:

Initially, a blood sample of 2ml was obtained from each participant and was assayed for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) by using kits manufactured by Randox®. AST/ALT ratio was calculated from the obtained AST and ALT values. Body mass index (BMI) of the participants was also calculated by measuring their body mass and height. After three months and six months from the initial study, only HIV-infected children who were receiving HAART were restudied for liver enzymes assay. Although, numerous liver enzymes, apart from ALT and AST, are used as markers of hepatotoxicity, all the enzymes may not be assayed routinely in a resource-limited country due to financial constraint. In the previous studies that assessed antiretroviral drug-related hepatotoxicity in pregnant women, ALT and AST were the only marker enzymes assayed.[18,19] We, therefore assayed only ALT and AST in this study.

Casualty such as death of participants during the periods of study was also documented.

Data analysis plan:

The measured outcomes such as ALT, AST, AST/ALT ratio and BMI of HIV-infected children on HAART were compared with other children by using ANOVA with IBM SPSS version 20 software package. Student t-test was also used to compare the mean results of group A or group D with each of the other five groups.

RESULTS

In the initial phase of the study, 238 participants comprising 118 (49.6%) boys and 120 (50.4%) girls were enrolled for the study. The participants were enrolled at a mean age of 27.3±23.7month with mean weight of 13.3±7.5kg. Out of 91 HIV-infected children aged 0-5 years on HAART who were enrolled for the initial study, 59 (64.8%) children, comprising of males (33) and females (26) were followed up after three months of the initial study. Fifty-six (61.5%) children comprising of males (32) and females (24) were followed up after six months of the initial study (Table 1).

The result of liver function test showed that liver enzyme ALT was highest in group A (12.8±11.0IU/L) followed by group C2 (11.7±20.7IU/L), group C3 (11.2±6.9IU/L), group C1 (10.9±7.8IU/L), group B (6.5±2.6IU/L) and group D (5.8±3.4IU/L) respectively. In Table 2, the mean value of ALT (p=0.005) and AST (p=0.013) varied significantly when all the six groups were compared using ANOVA. However, the AST/ALT ratio did not vary significantly (p=0.539) when all the six groups were compared. The mean values of ALT (p=0.000) and AST (p=0.009) of children in group A and group D vary significantly when group A and group D were compared using student t-test.

In Table 3, the mean values of the liver enzymes and their ratios after 3 and 6 months of follow up were compared with the initial values during the first phase of the study. Both the ALT and AST enzymes significantly decreased but their ratios were non-significantly increased during the follow up periods.

Table 1: Characteristics of study participants during first phase in southern Nigeria

Total number of children during first phase of study		**HIV-infected children on HAART**		
First phase		First Phase	Second phase	Third phase
Number of children	238	91	59	56
Male	118 (49.5%)	46 (50.5%)	33 (55.9%)	32(57.1%)
Female	120 (50.4%)	45 (49.5%)	26 (44.0%)	24 (42.8%)
Mean age (months)	27.3±23.7	44.8±20.5	50.5±18.6	52.9±20.6
Average Weight (Kg)	13.3±7.5	16.6±7.1	17.1±4.8	16.8±4.0
Body mass index (kg/m^2)	19.0±10.9	18.9±10.9	18.3±3.7	17.1±2.5
Mean baseline CD4 (cells/mm^3)	NA	945±548.2	897.1±503.0	999.2± 551.6
WHO Clinical Stage	NR	1	1	1

* It implies asymptomatic stage of HIV infection, NA=Not available, NR= Not relevant

Table 2: Impact of HAART on liver of study participants in southern Nigeria during first phase

Groups	Number of participants	Liver Function Test			Body Mass I ndex (BMI) (Kg/m^2)
		ALT (IU/L)	AST (IU/L)	Ratio	
A	91	12.8±11.0	23.9±27.3	3.1±6.1	18.9±10.9
C2	18	11.7±20.7	35.4±53.1	4.9±4.3	20.2±8.9
C3	48	11.2±6.9	24.9±15.9	3.0±2.4	24.1±9.3
C1	24	10.9±7.8	22.8±12.8	2.4±1.3	19.4±9.7
B	11	6.5±2.6	16.7±7.6	2.7±1.3	21.7±7.7
D**	46	5.8±3.4	12.4±6.8	2.9±2.6	17.1±2.8
p-vaue* (ANOVA)		p=0.005	p=0.013	p=0.539	p=0.140

*means of parameters in group A were compared with other groups. Groups were arranged in descending order of ALT values.
**Only participants in group D were exclusively breastfed for one year while others were exclusively breastfed for six months.

Table 3: Impact of HAART on liver of group A children on HAART in southern Nigeria

Phase	Number of participants	Liver function test		
		ALT (IU/L)	AST (IU/L)	RATIO
First (Initial study)	91	12.8±11.0	23.9±27.3	3.1±6.1
Three months later	59	7.8±6.1	16.7±8.6	3.4±3.2
Percentage change in liver enzyme		-39.1% (decreased)	-30.1% (decreased)	+9.7% (increased)
p-value		0.001	0.055	0.864
Six months later	56	6.3±3.9	16.2±8.7	3.7±3.9
Percentage change in liver enzyme		-50.8% (decreased)	-32.2% (decreased)	+19.4% (increased)
p-value		0.000	0.04	0.683

Death casualty:
Five (5.4%) of the HIV-infected children who were commenced on HAART regimens from the centres died. One of the deceased HIV-infected children was diagnosed with hepatomegaly before her death. Death was not associated with progression of HIV- infection and they were known to be adherent to therapy.

DISCUSSION
The initial phase of our study indicated that elevated liver enzyme, ALT, of HIV-infected children on HAART probably suggested mild liver injury. Previous studies had indicated that HAART was associated with liver injury in adults.[5,20] It was earlier reported in a study that incidence of severe liver injury and liver failure occurred among patients on HAART. The study reported that liver injury in patients on HAART was an emerging problem due to the frequency of liver injury and their potential adverse clinical outcome.[5] Elevated liver enzyme AST was very pronounced among HIV-exposed children on

co-trimoxazole at six months probably suggesting drug interaction between co-trimoxazole ingested by the children and HAART that were secreted in the breast milk of HIV-mothers on HAART. Previous study had indicated that sulphonamide and trimetoprim form additive toxicity with lamivudine and zidovudine causing elevated transaminases.[21] The administration of co-trimoxazole, at doses used for prophylactic treatment of *Pneumocystis carinii pneumonia* results in a 40% increase in lamivudine levels which is caused by trimethoprim. It was indicated that co-administration of lamivudine with high dose trimethoprim should be avoided.[21] It is a common practice in West Africa that HIV-mothers breastfeed their children exclusively for six months before introduction of meals while some mothers still breastfeed children till one year of age. Previous study indicated that among low-income HIV-infected mothers who are living in developing countries, exclusive breastfeeding was accepted and feasible as demonstrated by the high adherence rate. Early breastfeeding cessation was challenging due to maternal fears, household food insecurity and social stigma. Mothers learned to accept early weaning as HIV prevention strategy with consistent counselling and support. Exclusive breastfeeding for 6 months is widely promoted for optimal feeding within the general population of Malawi, regardless of HIV status, and thus carries no stigma. Southern Africa has also reported success using counselling sessions to promote exclusive breastfeeding. Early breastfeeding cessation at 4 months significantly increased the risk of growth faltering, severe morbidity and death.[22]

Six months after the initial study, both liver enzymes ALT and AST of the HIV-infected children on HAART were reduced probably suggesting lowering of the elevated aminotransferases. This observation is consistent with report of previous study in adults.[23] Mechanism of lowering of elevated aminotransferases induced by antiretroviral drugs was explained as: antiretroviral drugs potentiate the activation of death receptors or intracellular stress pathways. Hepatocytes promote mechanisms of cytoprotection against the oxidative stress caused by drug metabolism. Heat-shock proteins which are induced by various forms of stress including drugs exert cytoprotective functions thereby helping to tolerate potentially harmful toxicants. A rise in heat-shock proteins in individuals may help the liver adapt to and minimize drug toxicity. Anti-oxidation stress mechanisms also explain the spontaneous normalization in the levels of ALT and AST in spite of continuous HAART intake.[8]

In a previous study, it was reported that high and low levels of liver enzymes, ALT and AST concentrations after starting HAART may be related to adaptation phenomenon as liver enzymes normalize despite continuity of HAART.[10]

Though the cause of death of five HIV-infected children who received HAART regimen from the centres could not be ascertained, one of the deceased was diagnosed with hepatomegally. In a previous study, a mortality of 5.7% was reported among children on HAART.[24] The authors suggested that the higher mortality rates in poor economy countries during the early few months of HAART initiation, compared with developed countries, could be explained by the low CD4 cell counts, advanced clinical stage, prevalence of coexisting infection at the time of HAART initiation, occurrence of IRS in these patients with late-stage HIV infection and adverse reactions caused by antiretroviral drugs were responsible for morbidity and mortality. In our study all the participants on HAART were asymptomatic, which could be the reason for lower death rate experienced.

The limitation of the study included lack of screening of participants for confounders such as hepatitis C virus (HCV) and hepatitis B virus (HBV), inadequate liver function tests and restriction of access to the folders of deceased participants by the management of the hospitals.

CONCLUSION

This study indicated a significant association of HAART with lowering of alanine aminotransferase and aspartate aminotransferase after initial elevation in children of 0-5 years old.

Monitoring of HAART regimens for elevation of liver enzymes ALT and AST should be incorporated in the management of HIV infection in 0-5years old.

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REFERENCES

1. Aledort JE, Ronald A, Le Blancq SM, Ridzon R, Landay A, Rafael ME, Shea MV, Safrit J, Peeling RW, Hellmann N, Mwaba P, Holmes K and Wilfert C (2006). Reducing the burden of HIV/AIDS in infants: the contribution of improved diagnostics. *Nature* 444: S119-128.
2. Joint United Nations Programme on HIV/AIDS (2010). Overview of the global AIDS epidemic. *UN report on the global AIDS epidemic 2010*. ISBN 978-92-9173-871-7.

http://www.unaids.org/globalreport/Global_report.htm. Accessed March 14, 2014.

3. Palella FJ, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, Aschman DJ and Holmberg SD (1998). Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. *New England Journal of Medicine* 338: 853-860.
4. Lukas GM, Chaisson RE and Moore RD (1999). Highly active antiretroviral therapy in a large urban clinic: risk factors for virologic failure and adverse drug reactions. *Annals of International Medicine* 131: 81-87.
5. Puoti M, Torti C, Ripamonti D, Castelli F, Zaltron S, Zanini B, Spinetti A, Putzolu V, Casari S, Tomasoni L, Quiros-Roldan E, Favret M, Berchich L, Grigolato P, Callea F and Carosi G for the HIV-HCV co-infection Study Group (2003). Severe hepatotoxicity during combination antiretroviral treatment: incidence, liver histology and outcome. *Journal of acquired immune deficiency syndromes* 32: 259-267.
6. Nunez MJ, Martin-Carbonero L, Moreno V, Valencia E, Garcia-Samaniego J, Gonzalez-Castillo J, Barreiro P, Gonzales-Lahoz J and Soriano V (2006). Impact of antiretroviral treatment-related toxicities on hospital admissions in HIV-infected patients. *AIDS Research Human Retroviruses* 22: 825-829.
7. Bica I, McGovern B, Dhar R, Stone D, McGowan K, Scheib R and Snydman DR (2001). Increasing mortality due to end-stage liver disease in patients with HIV-infection. *Clinical Infectious Diseases* 32: 492-497.
8. Nunez M (2006). Hepatotoxicity of antiretrovirals: incidence, mechanisms and management. *Journal of hepatology* 44: S132-139.
9. World Health Organisation (2013). Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV-Infection. Recommendations for a public health approach. www.who.int. Accessed January 18, 2015.
10. Soriano V, Puoti M, Garcia-Gasco P, Rockstroh JK, Benhamou Y, Barreiro P and McGovern B (2008). Antiretroviral drugs and liver injury. *AIDS* 22(1): 1-3.
11. Kaplowitz N (2004). Drug-induced liver injury. *Clinical Infectious Diseases* 38(2): 44-48.
12. Raosoft Inc. 2004. Sample size calculator. Retrieved Aug. 30, 2015, from www.raosoft.com/samplesize.html. Accessed May 23, 2015.
13. Laurent M, Landreau-Mascaro A, Rekacewicz C, Berrebi A, Be´nifla JL, Burgard M, Lachassine E, Barret B, Chaix M, Bongain A, Ciraru-Vigneron N, Crenn-He´bert C, Delfraissy J, Rouzioux C and Mayaux M (2001). Lamivudine-Zidovudine combination for prevention of maternal-infant transmission of HIV-1. *Journal of American Medical Association* 285(16): 2083-2093.
14. Department of Health and Human Services (DHHS) (1991). Protection of human subjects. Code of Federal Regulations 45 CFR 46 Subpart D-Additional DHHS Protections for Children involved as Subjects in Research. (Department of Health and Human Services, National Institutes of Health, Office for Protection from Research Risks, Rockville, MD). Accessed June 18, 2014.
15. Laughon MM, Benjamin Jr DK, Capparelli EV, Kearns GL, Berezny K, Paul IM, Wade K, Barett J, Smith PB and Cohen-Wolkowiez M (2011). Innovative clinical trial design for pediatric therapeutics. *Expert Review of Clinical Pharmacology* 4(5):643-652.
16. Tabor E (2009). FDA requirements for clinical studies in pediatric patients. *Regulatory Focus* 14:16-21.
17. Melo-Martin I, Sondhi D and Crystal RG (2011). When ethics constrains clinical research: trial design of control arms in "greater than minimal risk" pediatric trials. *Human Gene Therapy* 22:1121-1127.
18. Ouyang DW, Shapiro DE, Lu M, Brogly SB, French AL, Leighty RM, Thompson B, Tuomala RE, and Hershow RC (2009). Increased risk of hepatotoxicity in HIV-infected pregnant women receiving antiretroviral therapy independent of nevirapine exposure. *AIDS* 23(18): 2425–2430.
19. Jamisse L, Balkus J, Hitti J, Gloyd S, Manuel R, Osman N, Djedje M, Farquhar C (2007). Antiretroviral-associated toxicity among HIV-1-seropositive pregnant women in Mozambique receiving nevirapine-based regimens. *Journal of Acquired Immune Deficiency Syndrome* 44(4): 371-376.
20. Ugiagbe RA, Malu AO, Bojuwoye BJ and Onunu AN (2012). Incidence of hepatotoxicity of highly active antiretroviral therapy in tertiary health center in Nigeria. *Nigeria postgradraduate Medical Journal* 19(3): 127-132.
21. Heylen R and Miller R (1997). Adverse effects of

medications commonly used in the treatment of adult HIV-positive patients: Part 2. *Genitourinary Medicine* 73: 5-11.

22. Parker ME, Bentley ME, Chasela C, Adair L, Piwoz EG, Jamieson DJ, Ellington S, Kayira D, Soko A, Mkhomawanthu C, Tembo M, Martinson F and van der Horst C (2011). The acceptance and feasibility of replacement feeding at 6 months as an HIV prevention method in Lilangwe, Malawi: results from the BAN study. *AIDS education and prevention* 23(3): 281-285.
23. Nunez M and Soriano V (2005). Hepatotoxicity of antiretrovirals: Incidence, mechanisms and management. *Drug safety* 281: 53-66.

Puthanakit T, Aurpibul L, Oberdorfer P, Akarathum N, Kanjananit S, Wannarit P, Sirisanthana T and Sirisanthana V (2007). Hospitalization and mortality among HIV-infected children after receiving HAART. *HIV/AIDS Clinical Infectious Diseases* 44: 599-604.