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EFFECTS OF HAART ON ORGANS OF CHILDREN BORN TO HIV-INFECTED MOTHERS IN SOUTHSOUTH NIGERIA

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ABSTRACT

Provision of antiretroviral prophylaxis to pregnant women living with HIV has prevented more than 350,000 children from acquiring HIV infection since 1995. Aim of the study is to detect the effect of HAART on kidney and liver of children aged 0-5 years in South-south Nigeria. The study was granted ethical approval by University of Uyo Teaching Hospital and Emmanuel General Hospital Eket. Fifty children were enrolled for the study after consent was given by their care-givers. They were divided into four groups. The first group consisted of asymptomatic HIV infected children receiving HAART combination, 3TC+NVP+ZDV. The second group consisted of exposed children who received nevirapine for six weeks and later continued with cotrimoxazole. The third group consisted of HIV positive children with uncompromised immunity receiving cotrimoxazole. The fourth group comprised the control children who did not receive any drug. A 2ml blood was collected from each participant and separated to obtain serum. Sera were analyzed with Randox ALT, AST and creatinine kits. The result showed that seventeen participants (50%) in Group I had elevated liver enzyme ALT while two participants (20%) had elevated ALT in Group II and none among the control children had elevated ALT. Twenty-eight (82.35%) participants had elevated liver enzyme AST in

Group I, eight participants (80%) in Group II and three (60%) participants in Group IV. Fourteen (41.18%) boys and six girls (17.65%) in Group I had abnormal serum creatinine values, Four boys (40%) and four girls (40%) in Group II and two (40%) boys in Group IV also had abnormal serum creatinine value. Sixteen (47.06%) boys and eleven (32.35%) girls had abnormal creatinine clearance in Group I, four (40%) boys and five (50%) girls had abnormal creatinine clearance in Group II. Two (40%) boys and two (40%) girls had abnormal creatinine clearance in Group IV. Statistical analysis of variance at confidence interval of 90%, ALT ($p = .073$) and serum creatinine ($p = .095$) were significantly higher in Group I than Group IV.

The study showed that HAART combination had significant severe effect on liver and kidney function of children from zero to five years.

Keywords: Liver, Kidney, HAART, ALT, AST, Creatinine, Antiretroviral.

INTRODUCTION

At the start of 2011, an estimated 3.4 million children below 15 years were living with HIV globally. There were 390,000 new infections in children below 15 years. About 250,000 children below 15 years died from AIDS- related causes (WHO, 2011).

Providing antiretroviral prophylaxis to pregnant women living with HIV has prevented more than 350,000 children from acquiring HIV infection since 1995. Eighty-six percent of the children who avoided infection live in sub-Saharan Africa, the region with the highest prevalence of HIV infection in women of reproductive age (WHO, 2011).

As access to services for preventing the mother to child transmission of HIV increased, the annual number of children acquiring HIV infection stabilized in the beginning of 2000s and later decreased sharply in the past few years. The number of children below 15 years living with HIV globally has leveled off in the past few years and reached 3.4 millions in 2010, more than 90% were living in sub-Saharan Africa (UNAIDS, 2010).

All infants born to HIV-infected mothers should receive antiretroviral prophylaxis (WHO, 2010). This involves both the short postpartum prophylaxis for 6 weeks recommended for all HIV- exposed infants despite regimen used for preventing mother to child transmission/ breastfeeding and extended antiretroviral therapy or other antiretroviral medicine for the mothers or infants during breastfeeding. (WHO, 2011) Laurent et al reported that there was severe adverse effect with use of lamivudine- zidovudine combination among children (Laurent et al, 2001).

Although HAART has been shown to improve renal function, long term use may be associated with significant nephrotoxicity, especially tenofovir and releated nucleotide analogs (Madeddu, 2006; Rho 2007). Also, hepatic steatosis and abnormal liver function tests are common side effects of several ARTs (Nunez, 2010; Ugiagbe, 2012).

METHOD

Fifty (50) children participated in this study in South-south Nigeria after ethical approval was received from University of Uyo Teaching Hospital, Uyo and Emmanuel General Hospital, Eket to conduct the study. The study was commenced in the HIV Paediatric Clinic and Children Outpatient Clinic of the Hospitals. Parents/ Guardians/ caregivers of HIV infected children, exposed children and normal children were informed of the study. Consent forms were given to the care givers after acceptance to allow their children to participate. A one-on-one discussion was held with each care giver. The recruited children were assigned to four groups. Group I consisted of HIV infected children who have started Highly Active Antiretroviral Therapy (HAART). They took Nevirapine + Zidovudine + Lamivudine combination (NVP+ZDV+3TC). Group II consisted of children that were born to HIV infected mothers whose first result at six weeks and six months of life showed that they were seronegative. Groups III consisted of confirmed HIV infected children with higher CD4 count and were not eligible to start HAART at age more than 2 years. Group IV consisted of children that were used as control. They did not have HIV infection and were not on long term drug regimen. They did not have any neurological or endocrinological problem. 2ml blood sample was collected from each of the recruited children. The blood samples were sent to the laboratory for blood chemistry analysis. The blood samples were centrifuged to obtain serum. The sera were used. Blood chemistry was done on two liver enzymes namely alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and on creatinine to evaluate renal function.

Alanine Aminotransferase (ALT) (Randox)

- Sample: Serum
- Reagent: Buffer (Phosphate buffer 100mmol/l, pH 7.4, L-alanine 200mmol/l, α-oxoglutarate 2.0mmol/l) and 2,4-dinitrophenylhydrazine 2.0mmol/l.
- Wavelength: Hg 546nm
- Cuvette: 1cm light path
- Incubation temperature: 37^0C

Using measurement against sample blank

0.5ml of buffer was pipette into a test tube without serum as the sample blank. 0.5ml of buffer and 0.1ml of serum was pipette into another test tube known as the sample. Each of the test tubes was mixed and incubated for exactly 30minutes at 37^0C. Pipette was used to add 0.5ml of 2,4-dinitrophenylhydrazine and 0.1ml of sample into the sample blank tube. 0.5ml of 2, 4-dinitrophenylhydrazine was added into the sample tube with the use of pipette. Each of the tubes was mixed and allowed to stand for exactly 20minutes at 25^0C. Then, 5.0ml of Sodium Hydroxide was added both into the sample blank tube and the sample tube. Each tube was mixed and the absorbance of the sample tube was read against the sample blank tube after 5 minutes.

Similar procedure was used for AST and Creatinine. The absorbance of the sample was read against the sample blank after 5 minutes. The absorbance was compared with established values from the manufacturer's kit table. The obtained serum creatinine value was used to calculate creatinine clearance using Schwartz equation as shown below:

$$\text{CrCl (ml/min/1.73m}^2\text{)} = \frac{\text{length (cm) X k}}{\text{Serum creatinine}}$$

K= 0.45 for infants 1 to 52 weeks old

K= 0.55 for children 1 to 13 years old (Schwartz et al., 1976, Schwartz et al., 1984, Haenggi 1999,)

RESULT

Among the fifty study participants aged zero to five years, there were twenty-eight (56%) male participants and twenty-two (44%) female participants. The average age of study participants was 30.44 months and their average weight was 13.53 kg. The body mass index (BMI) of the study participants in their respective groups were 16.94kg/m^2, 21.54kg/m^2, 17.33kg/m^2 for groups I, II and IV respectively. The mean baseline CD4 cell for study participants was 1031 cells/mm^3 (Table 1).

In Group I, the result showed that seventeen (50%) children had normal values of liver enzyme ALT while seventeen (50%) had elevated value of ALT. Six (17.65%) children had normal value of liver enzyme AST while twenty-eight (82.35%) had elevated AST values. Six (17.65%) of the children had normal AST/ALT ratio while twenty-eight (82.35%) had elevated ratio. Six (17.65%) boys had serum creatinine values within the normal range of 53-97µmol/l while fourteen (41.18%) boys had abnormal value. Eight (23.52%) girls had

creatinine values in the normal range of 44-80µmol/l while six (17.65%) had abnormal values. Four (11.77%) boys had normal creatinine clearance within normal range of 97-137ml/min/1.732m^2 while sixteen (47.06%) boys had abnormal creatinine clearance. Three (8.82%) girls had creatinine clearance in the normal range of 88-128ml/min/1.732m^2 while eleven (32.35%) had abnormal creatinine clearance (Table 2).

In Group II, eight children (80%) had normal values of ALT while two (20%) had elevated ALT values. Two (20%) had normal values of AST while eight (80%) had elevated values of AST. Two (20%) of the children had normal AST/ALT ratio of 1.0 while eight (80%) had more than 1.0. None of the boys in this group had serum creatinine values in the normal range of 53-97µmol/l while four (40%) boys had abnormal serum creatinine values. Two (20%) girls had serum creatinine values in the normal range of 44-80µmol/l while four (40%) had abnormal serum creatinine values. None of the boys (0%) had normal value of creatinine clearance (97-137ml/min/1.732m^2) while four boys (40%) had abnormal creatinine clearance. One (10%) girl had normal craetinine clearance (88-128ml/min/1.732m^2) while five (50%) girls had creatinine clearance had abnormal creatinine clearance (Table 2).

In Group III, there was only one recruited participant who had normal values of ALT. AST value and AST/ALT ratio were above normal values. The serum creatinine and creatinine clearance values of the boy are outside the normal range of 53-97µmol/l and 97-137ml/min/1.732m^2 respectively (Table 2).

In Group IV, five (100%) children had normal ALT values. Two (40%) children had normal AST values while three (60%) had elevated AST values. All (100%) the children had AST/ALT ratio greater than 1.0. One (20%) boy had serum creatinine value in the normal range of 53-97µmol/l while two (40%) boys had abnormal serum creatinine value. Two (40%) girls had serum creatinine values in the normal range of 44-80µmol/l. Two (40%) boys and two (40%) girls had abnormal creatinine clearance (Table 2).

The liver function test revealed that liver enzyme ALT mean value was lowest in Group IV (7.63IU/L), followed by Group II (8.88IU/L), Group 3 (10.4IU/L) and highest in Group I (15.26IU/L). The liver enzyme AST mean value was lowest in Group IV (17.66 IU/L) followed by Group III (18.05IU/L), Group II (19.40) and highest in Group I (26.19IU/L) (Table 3).

The kidney function test showed that Group IV (64.85µmol/l) had highest mean values which were within the normal range of creatinine value for male (53-97µmol/l) and female (44-80µmol/l) followed by Group I (50.07µmol/l). Both Group II (38.19µmol/l) and Group III (40.71µmol/l) had lowest mean values which were below normal range for both male and female. The creatinine clearance mean value for Group IV (70.49ml/min/1.732m^2) was lowest followed by Group II (71.77ml/min/1.732m^2) and Group III (81.32ml/min/1.732m^2). It was highest for Group I (82.77ml/min/1.732m^2). None of the group mean values for creatinine clearance fall within normal range for male (97-137ml/min/1.732m^2) and female (88-128ml/min/1.732m^2) (Table 3).

The statistical analysis of variance was used with IBM SPSS version 20 software to compare the means of the groups on ALT, AST, serum creatinine and creatinine clearance. The obtained p-values at 95% confidence interval were as shown ALT (.073), AST (.511), serum creatinine (.095) and creatinine clearance (.507). Body mass index p-value was .498.

Total number of participants: 50

Table 1: Basic characteristics of study participants

Characterstics	**N= 50**
Male	28 (56%)
Female	22 (44%)
Mean age (months)	30.44±21.74
Average Weight (Kg)	13.53±5.25
BMI (I)	16.94±3.95
BMI (II)	21.54
BMI (III)	-
BMI (IV)	17.33±1.80
Mean baseline CD4 (cells/mm^3)	1031±585.18
Stage	1

Table 2: Comparison of Parameters With Normal Standard

GROUPS	ENZYMES	COMPARISON WITH MANUFACTURER' (Randox) NORMAL STANDARD	FREQUENCY	PERCENTAGE
I	ALT	≤12 IU/L	17	50%
		>12 IU/L	17	50%
	AST	≤12 IU/L	6	17.65%
		>12 IU/L	28	82.35%
	AST/ALT	≤1	6	17.65%
		>1	28	82.35%
	Male Creatinine	53- 97 µmol/l	6	17.65%
		Outside normal range	14	41.18%
	Female Creatinine	44-80 µmol/l	8	23.52%
		Outside Normal range	6	17.65%
	Male Creatinine Clearance	97- 137ml/min/1.732m^2	4	11.77%
		Outside normal range	16	47.06%
	Female creatinine clearance	88- 128ml/min/1.732m^2	3	8.82%
		Outside normal range	11	32.35%
	Total Number of Participants		**34**	
II	ALT	≤12 IU/L	8	80%
		>12 IU/L	2	20%
	AST	≤12 IU/L	2	20%
		>12 IU/L	8	80%
	AST/ALT	≤1	2	20%
		>1	8	80%
	Male Creatinine	53- 97 µmol/l	0	0%
		Outside normal range	4	40%
	Female Creatinine	44-80 µmol/l	2	20%
		Outside normal range	4	40%
	Male Creatinine Clearance	97- 137ml/min/1.732m^2	0	0%
		Outside normal range	4	40%
	Female creatinine clearance	88- 128ml/min/1.732	1	10%
		Outside normal range	5	50%
	Total Number of Participants		**10**	
III	ALT	≤12 IU/L	1	100%
		>12 IU/L	0	0%
	AST	≤12 IU/L	0	0%
		>12 IU/L	1	100%
	AST/ALT	≤1	0	0%
		>1	1	100%
	Male Creatinine	53- 97 µmol/l	0	0%
		Outside normal range	1	100%

	Female Creatinine	44-80 µmol/l	0	0%
		Outside normal range	0	0%
	Male Creatinine Clearance	97-137ml/min/1.732m^2	0	0%
		Outside normal range	1	100%
	Female creatinine clearance	88- 128ml/min/1.732m^2	0	0%
		Outside normal range	0	0%
	Total number of Participants		**1**	
IV	ALT	≤12 IU/L	5	100%
		>12 IU/L	0	0%
	AST	≤12 IU/L	2	40%
		>12 IU/L	3	60%
	AST/ALT	≤1	0	0%
		>1	5	100%
	Male Creatinine	53- 97 µmol/l	1	20%
		Outside normal range	2	40%
	Female Creatinine	44-80 µmol/l	2	40%
		Outside normal range	0	0%
	Male Creatinine Clearance	97-137ml/min/1.732m^2	0	0%
		Outside normal range	2	40%
	Female creatinine clearance	88-128ml/min/1.732m^2	0	0%
		Outside normal range	2	40%
	Total number of Participants		**5**	

Table 3: Liver function and kidney function tests

GROUPS	Liver function test (mean±SD)		Kidney function test (mean±SD)	
	ALT (IU/L)	AST (IU/L)	Creatinine (µmol/L)	Creatinine clearance (ml/min/1.732m^2)
I (n=34)	15.26±9.04	26.19±18.34	50.07±18.66	82.77±41.70
II (n=10)	8.88±6.32	19.40±7.41	38.19±18.28	71.77±39.00
III (n=1)	10.40	18.05	40.71	81.32
IV (n=5)	7.63±0.98	17.66±9.95	64.85±26.22	70.49±54.59

ALT (p=.073), AST (p=.511), creatinine (p=.095), creatinine clearance (p=.507)

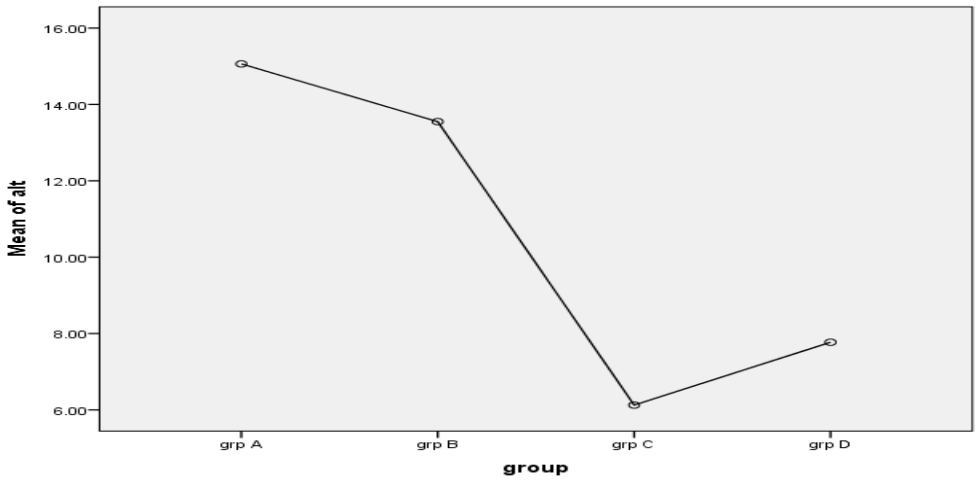

Fig 1: Comparison of alanine aminotransferase among groups

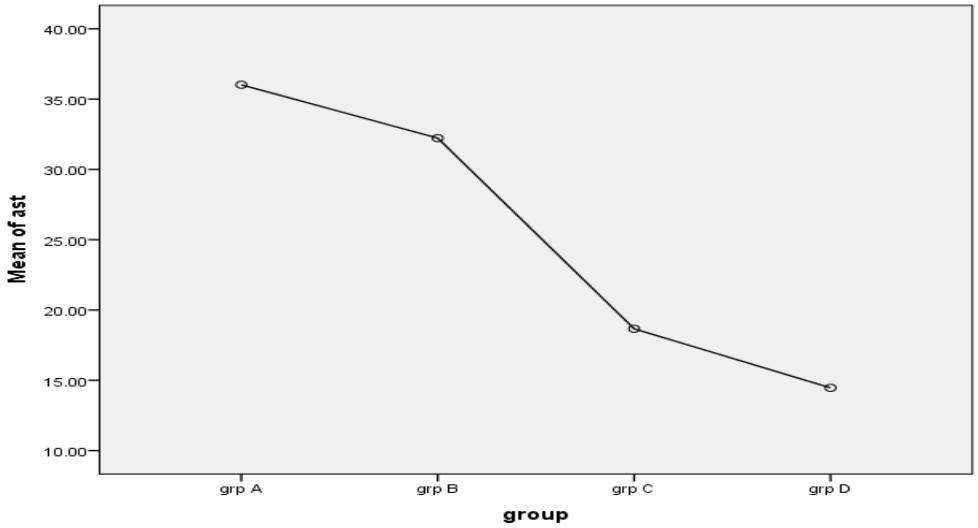

Fig 2: Comparison of aspartate aminotransferase among groups

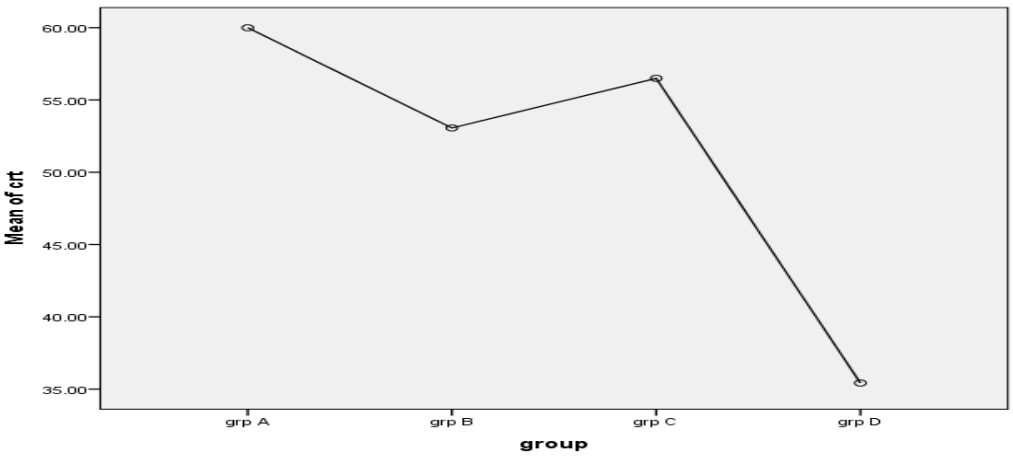

Fig 3: Comparison of serum creatinine among groups

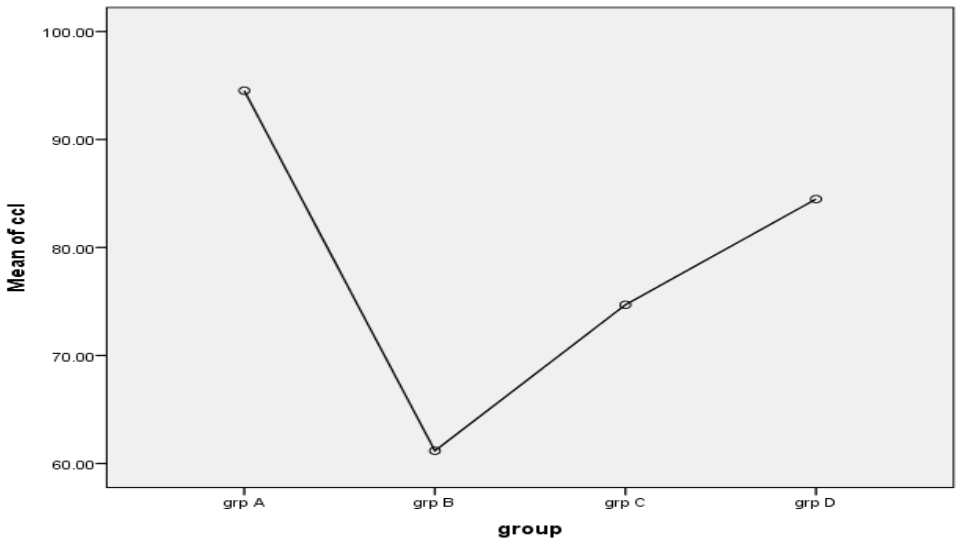

Fig 4: Comparison of creatinine clearance among groups

DISCUSSION

Children receiving HAART combination medicines had less BMI than the control children while those children receiving single dose nevirapine and co-trimoxazole were having BMI greater than that of the control children.

The percentage of children with elevated liver enzyme ALT among the groups follows a decreasing order as there were fifty percent of children on HAART had ALT elevation, twenty percent of children on prophylactic single dose nevirapine and co-trimoxazole while there was no child with elevated liver enzyme ALT in the control Group. This showed a marked difference in the effect of HAART on liver of children on HAART over the control children. This supported earlier research done by Laurent et al (2001).

In a similar pattern, more than eighty percent of the children on HAART and eighty percent of children on initial nevirapine with continuous co-trimoxazole had elevated liver enzyme AST which is an indicator of liver injury. Sixty percent of the control children had elevated AST. This indicated a marked difference in the effect of HAART on liver enzymes to those of the control children (Nunez, 2010; Ugiagbe et al., 2012).

More than eighty percent of children on HAART and eighty percent of children who took nevirapine and later continued on co-trimoxazole AST/ALT ratio greater than 1.0 while all the children in the control Group had AST/ALT ratio greater than 1.0. This ratio was bad for both the control children and other study participants. It was assumed that the control

children could have liver injury due to undisclosed use of drugs like herbal remedy (Nunez, 2010; Ugiagbe et al., 2012).

Fifty percent of the children on HAART had abnormal serum creatinine, while eighty percent of the children who initially took nevirapine and later continued with co-trimoxazole had abnormal serum creatinine. Only forty percent of the control children had abnormal serum creatinine. Therefore, there was an indication that more children who took HAART combination and those who took prophylactic nevirapine and later continued with co-trimoxazole had severe effect on renal function than control children (Madeddu et al., 2006; Rho et al., 2007).

About eighty percent of the children on HAART had abnormal creatinine clearance while ninety percent of the children who took initial nevirapine and continued with co-trimoxazole and eighty percent of the control children had abnormal creatinine clearance. There was not much difference in the creatinine clearance of these study participants. It was therefore assumed that the control children did not have enhanced kidney function like those children on HAART. More of the children on single dose nevirapine prophylaxis and co-trimoxazole seemed to have more severe effects on kidney function than the children that received HAART and the control children (Madeddu et al., 2006; Rho et al., 2007).

Considering the group mean value for liver enzyme ALT, children on HAART was highest with a value twice the mean value of control children. Similarly, the mean value of liver enzyme AST of children on HAART was one and a half times the mean value of control children. This confirmed that HAART had severe effect on liver enzymes ALT and AST which indicated the presence of liver injury (Nunez 2010, Ugiagbe 2012).

The mean value for serum creatinine was highest for the control children and fall within the normal range of 44-80µmol/l for female and 53-97µmol/l for male. The mean value for serum creatinine among exposed children on prophylactic nevirapine and co-trimoxazole and HIV-infected children on cotrimoxazole were below the normal range for both male and female. Both children on HAART combination and exposed children on single dose nevirapine prophylaxis and co-trimoxazole had mean value of creatinine clearance greater than that of the control group. The HIV-infected children on co-trimoxazole also had creatinine clearance value greater than the control children. Though all the groups had mean value of creatinine clearance below the normal value for male and female, children on HAART, exposed

children on nevirapine and co-trimoxazole and HIV-infected children on co-trimoxazole had improved kidney function over the control children (Madeddu, 2006; Rho, 2007).

At confidence interval of 90%, HAART combination showed statistically reliable effect on liver (ALT) and kidney (serum creatinine).

In conclusion, it is very important to acknowledge that many of the children who took HAART combination and antiretroviral drug nevirapine and co-trimoxazole are likely to experience significant effect on the liver and renal function from the impact of continuous use of HAART without drug therapeutic monitoring.

It was hereby recommended that policy makers in Government, State and Federal ministries of health should support inclusion of Drug Therapeutic Monitoring in the management of HIV/AIDS among children.

REFERENCES

1. WHO, UNAIDS, UNICEF. Global HIV/AIDS Response Epidemic Update and Health Sector Progress Towards Universal Access.2011.
2. UNAIDS and World Bank. New Infections by mode of Transmission in West Africa: A multicountry Analysis. March. Dakar, UNAIDS Regional Support Team for West and Central Africa.2010. www.unaids.org
3. WHO. Antiretroviral drugs for treating pregnant women and preventing HIV infection in infants: Recommendations for a Public Health approach. 2010. http://whqlibdoc.who.int/publications/2010
4. Laurent M, Aline L, Claire R, Alain B, Jean L, Marianne B, Eric L, Beatrice B, Marie-Laurie C, Andre B, Nicole C, Catherine C, Jean-Francois D, Christine R, Marie-Jeanne M, Stephanne B. (2001) Lamivudine-Zidovudine Combination for Prevention of Maternal-Infant Transmission of HIV-1. JAMA, 285(16): 2083-2093.
5. Madeddu G, Quirino T, Carradori S, Ricci E, Grosso C, Penco G, et al. (2006): Renal toxicity of HIV-infected patients receiving HAART including Tenofovir. Infez Med; 14(3): 125- 34.
6. Rho M, Perazella MA (2007). Nephrotoxicity associated with antiretroviral in HIV–infected patients. Curr Drug Saf. 2(2): 147- 54
7. Núñez M (2010). Clinical syndromes and consequences of antiretroviral-related hepatotoxicity. Hepatology. 2010; 52(3):1143-55. doi: 10.1002/hep.23716.

8. Ugiagbe RA, Malu AO, Bojuwoye BJ, Onunu AN (2012). Incidence of hepatotoxicity of highly active antiretroviral therapy in a tertiary health centre in Nigeria. NigerPostgrad Med J. 19(3):127-32.
9. Schwartz GJ, Haycock GB, Edelmann CM, Splitzer A. A simple estimate of glomerular Filtration Rate in children derived from body length and plasma creatinine. Pediatrics 1976; 58(2): 259-263.
10. Schwartz GJ, Feld LG, Langford DJ. A simple estimate of Glomerular Filtration rate in full term infants during the first year of life. The Journal of Pediatrics 1984; 104 (6): 849-854.
11. Brion LP, Fleischman AR, McCarton C, Schwartz GJ,. A simple estimate of glomerular filtration rate in low birth weight infants during the first year of life, noninvasive assessment of body composition and growth. The Journal of Pediatrics 1986; 109(4): 698-707.
12. Haenggi MH, Pelet J, Guignard JP. Estimation of glomerular filtration rate by the formula: GFR & #61; k x T . archives de Pediatrie 1999; 6(2): 165-172.
13. Schwartz GJ, Munoz A, Schneider MF et al. New equations to estimate GFR in children with CKD. Journal of American Society of Nephrology 2009; 20(3): 629-637.