World Journal of Pharmaceutical research

Volume 2, Issue 6, 3296-3311. Research Article ISSN 2277 – 7105

PRELIMINARY STUDY ON EFFECT OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) ON LIVER AND KIDNEYS OF CHILDREN BORN TO HIV INFECTED MOTHERS IN SOUTHWEST NIGERIA

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Article Received on 06 Sept. 2013,

Revised on 29 Sept. 2013, Accepted on 30 October 2013

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ABSTRACT

There are nearly 1200 daily new infections in children less than fifteen years of age, more than 90% of them found in low income countries. More than 275,000 children received ART, which made about 38% of those in need of ART. The aim of this study was to evaluate the effect of HAART on liver and kidneys of children from age zero to five years. One hundred and twenty-one children attending HIV clinic in University College Hospital, Ibadan were recruited for the study in four groups. The first group involved children who took HAART, the second involved children that took nevirapine for six weeks and continued with co-trimoxazole, the third group involved HIV infected children who were not on HAART but received co-trimoxazole. The fourth group involved the control children. A 2ml blood was collected from the femoral vein, centrifuged to obtain serum which was analyzed for liver enzymes namely ALT and AST and creatinine. Elevation of ALT was highest among group B (26.67%) followed by Group A (25%) and Group D (11.76%). Elevation of AST was highest among Group B (86.67%) followed by Group A (75%) and Group D (41.18%). Group B (62.22%) has the highest percentage of children with abnormal values for

serum creatinine followed by Group A (45%) and Group D (35.29%). Highest number of participants with abnormal creatinine clearance values was found in Group B (88.89%) followed by Group A (75%) and Group D (61.76%). Group 3 was not used in the comparison because only two participants represented the Group with contrasting results. Analysis of variance showed that HAART use has reliable effect on the kidneys ($p < .1$) and liver of children ($p < .000$).

Keywords: ART, HAART, HIV, Pediatrics, Children, liver, Kidney, AIDS.

INTRODUCTION

A total of 80,000 cases of HIV infection were reported in UK by June 2006 and 21,000 of the infected persons had acquired immunodeficiency syndromes (AIDS) of which eighty percent (80%) died. WHO report estimated that 38.6 million adults and 2.3million children worldwide were living with HIV at the end of 2005 (Ritter et. al., 2008). An estimated 430,000 new HIV infections occurred globally in infants and children, of which 90% were acquired through mother to child transmission (MTCT) (WHO, 2009). Almost 276,000 children globally received ART in 2008 (Gilks, 2006). WHO guidelines on use of ART in infants and children are based on a Public Health Approach to HIV care (UNICEF, 2009). These guidelines are harmonized with the treatment guidelines adopted for adults, pregnant women and for prevention of mother to child transmission (PMTCT) (WHO, 2009). Emphasis has been made that infants and children less than 18months of age who have been exposed to HIV should be closely monitored and should benefit early in life from child survival interventions, co-trimoxazole prophylaxis and antiretroviral therapy, even in the absence of virological testing (WHO, 2010).

Toxicity can be monitored clinically, based on child/ guardian reporting, physical examination and laboratory tests base on antiretroviral therapy in use and the standard of the healthcare provider. The decision to substitute a new antiretroviral drug is base on the severity of adverse reaction (WHO, 2009). 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 et al 2006, Rho et al., 2007]. Also, hepatic steatosis and abnormal liver function tests are common side effects of several ARTs [Ugiagbe et al., 2012, Nunez M, 2010].

This study monitors the effect of HAART on liver and kidneys of HIV-infected children.

MATERIALS AND METHOD

Materials:

- Blood samples
- Plain vacuum tubes (Silver Health Diagnostics^{R})
- Syringes & needles
- Disposable storage containers
- Disposable gloves
- Randox kits (ALT, AST, Creatinine)
- Centrifuge
- UNISPEC 23D Spectrophotometer (Surgifriend Medicals, England)
- Weighing balance
- Tape rule
- Methylated spirit
- Cotton wool
- Refrigerator

Research Centers

- Human Immunodefficiency Virus Pediatric Clinic,
  University College Hospital, Ibadan. Nigeria.
- Institute of Child Health, University College Hospital
- Children Outpatient Clinic, University College Hospital

Method

There were one hundred and twenty-one (121) children aged between zero and five years who participated in this research in Southwestern Nigeria after ethical approval was granted in University College Hospital, Ibadan to conduct the study. The study was commenced in the HIV Paediatric Clinic, Institute of Child Health and Children Outpatient Clinic of the Hospital. Parents/ Guardians of HIV infected children, exposed children and normal children were informed of the study. Consents were sought from the care givers. The volunteer care givers were given Informed consent to fill for their participating children/ wards. Discussion was held with each care giver. The recruited male and female children were assigned to four groups. Group A consisted of asymptomatic, HIV infected children who have started Highly Active Antiretroviral Therapy (HAART) in Stage 1 for more than three months. Group B consisted of children that were born to HIV infected mothers whose result at six weeks and

six months of life showed that they were HIV seronegative. Groups 3 consisted of confirmed HIV infected children with higher CD4 count and were not eligible to start HAART at age more than 2 years. Group 4 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. Each blood sample was spun to obtain serum from the blood. The sera were used. Blood chemistry was done on two liver enzymes namely alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and on creatinine and creatinine clearance 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. Creatinine clearance was calculated from serum creatinine by using:

Schwartz equation

CrCl (ml/min/1.73m2)= [length (cm) x k] / Scr [Schwartz et al., 1976]

K= 0.45 for infants 1 to 52 weeks old

K= 0.55 for children 1 to 13 years old

Shull equation

Crcl (ml/min/1.73m2) = ((0.035 x age) + 0.236) x 100)/ Scr [Shull et al., 1978]

(Serum creatinine was measured in µmol/l. Conversion of µmol/l to mg/dl was done by multiplying the value of creatinine with 88.4).

Descriptive analysis and statistical analysis of variance (ANOVA) were used to determine the effect among the groups.

RESULT

One hundred and twenty-one children aged 0-5 years participated in the study which made up of 55 (45.45%) males and 66 (54.55%) females. The average age of study participants was 26.64months with average weight of 11.21kg. The Body Mass Index (BMI) of participants in the groups were A (15.46kg/m^2), B (16.84kg/m^2), C (17.72kg/m^2) and D (16.98kg/m^2). The average CD4 cell of participants in Group A is 1073.4 cells/mm^3 (Table1).

Twenty-one (52.5%) of the HIV- infected children on HAART combination received zidovudine, lamivudine and nevirapine combination (AZT + 3TC + NVP), six (15%) received abacavir, lamivudine and lopinavir with ritonavir combination (ABC+3TC+LPV/RTV), five (12.5%) received efavirens, zidovudine and lamivudine combination (EFV + AZT + 3TC), three (7.5%) were on stavudine, lamivudine and nevirapine combination (D4T + 3TC + NVP) and another three (7.5%) were on zidovudine, lamivudine and lopinavir with ritonavir combination (AZT + 3TC + LPV/RTV) and two (5%) received abcavir, lamivudine and zidovudine combination (ABC + 3TC + AZT). The children on ABC+ 3TC + AZT had the highest BMI mean value (17.68kg/m^2), followed by those children on AZT + 3TC + LPv/RTv (16.94kg/m^2), EFV + AZT + 3TC (15.95kg/m^2), D4T + 3TC + NVP (15.60kg/m^2), AZT + 3TC + NVP (15.21kg/m^2) and least for children who took ABC + 3TC + LPv/RTv (14.40kg/m^2)(Table 2).

A comparison of the blood chemistry result of the research participants showed that in Group A, thirty (75%) participants had normal result for ALT, 10 (25%) participants had elevated ALT. For AST, ten (25%) participants had normal result, while thirty (75%) had elevated

AST. The AST/ALT ratio showed that six (15%) participants had normal values while thirty-four (85%) had elevated ratios. Creatinine result showed that, ten (25%) male participants had normal values; nine (22.5%) male participants had elevated creatinine. Eleven (27.5%) female participants had normal creatinine while nine (22.5%) of them had elevated creatinine. The creatinine clearance result showed that three (7.5%) male participants had normal creatinine clearance while sixteen (40%) male participants had abnormal creatinine clearance. Six (15%) female participants receiving HAART combination had normal creatinine clearance while fourteen (35%) of the female participants in the group had abnormal creatinine clearance (Table 3).

In Group B, thirty-three (73.33%) participants had normal result for ALT while twelve (26.67%) participants had elevated ALT. Six (13.33%) participants had normal AST result while thirty-nine (86.67%) had elevated AST. Five (11.11%) participants had normal AST/ALT ratio and forty (88.89%) participants had elevated AST/ALT ratio. Creatinine result showed that four (8.89%) of the male participants had normal creatinine while fourteen (31.11%) had abnormal creatinine. Thirteen (28.89%) of the female participants had normal creatinine and fourteen (31.11%) female participants had abnormal values. Creatinine clearance result showed that three (6.67%) male participants had normal creatinine clearance and fourteen (31.11%) had abnormal clearance values. Two (4.44%) female participants had normal creatinine clearance while twenty-six (57.78%) female participants had abnormal creatinine clearance values (Table 3).

In Group C, there were only two male research participants whose ALT values are normal. There was one (50%) normal and one (50%) elevated value for both AST and AST/ALT ratio. One (50%) male participant had normal creatinine value while the other one (50%) had elevated creatinine value. The two (100%) male participants had abnormal creatinine clearance (Table 3).

In Group D, thirty (88.24%) of the control participants had normal ALT values while four (11.76%) participants had elevated ALT. Twenty (58.82%) participants had normal AST and fourteen (41.18%) had elevated AST. Five (14.71%) of the participants in the control group had normal AST/ALT ratio while twenty-nine (85.29%) had elevated AST/ALT ratio. Eleven (32.35%) male control participants had normal creatinine value while five (14.7%) had abnormal creatinine values. Ten (29.41%) control female participants had normal creatinine values and seven (20.59%) female participants had abnormal creatinine values. One (2.94%)

male participants had normal creatinine clearance, eleven (32.35%) male participants had abnormal creatinine clearance, three (8.82%) female participants had normal creatinine clearance and ten (29.41%) had abnormal creatinine clearance (Table 3).

The group mean value for ALT was highest for group B (9.99IU/L) followed by group A (9.90IU/L), group C (6.32IU/L) and least for the control group D (5.2IU/L). The group mean value for AST was highest among participants in group B (23.55IU/L), followed by group A (16.84IU/L), group D (11.23IU/L) and least for group C (8.54IU/L). The creatinine mean value was highest for group D (59.18µmol/l) followed by group A (54.22µmol/l), group C (52.44µmol/l) and group B (44.22µmol/l) respectively. The creatinine clearance mean value was highest for group A (117.91ml/min/1.732m^2) followed by group D (82.58ml/min/1.732m^2), group B (74.64ml/min/1.732m^2) and group C (71.15ml/min/1.732m^2) respectively (Table 4).

The statistical analysis of variance was used with IBM SPSS version 20 software and the following p-values were obtained ALT (.000), AST (.000), serum creatinine (.060) and creatinine clearance (.086).

Table 1: Basic characteristics of participants

Characterstics	N= 121
Male	55 (45.45%)
Female	66 (54.55%)
Mean age (months)	26.64±24.18
Average Weight (Kg)	11.21±5.08
BMI (A)	15.46±1.65
BMI (B)	16.84±6.57
BMI (C)	17.72
BMI (D)	16.98±3.49
Mean baseline CD4 (cells/mm^3)	1073.4±1575.24
Stage	1

Table 2: HAART combinations used among research participants

S/N	HAART COMBINATIONS	NUMBER OF PARTICIPANTS	PERCENTAGE	BMI OF PARTICIPANTS WHO TOOK HAART COMBINATION(Kg/m^2)
1	ABC+ 3TC + LPv/rtv	6	15%	14.40
2	D4T + 3TC + NVP	3	7.5%	15.60
3	AZT + 3TC + NVP	21	52.5%	15.21
4	EFV + AZT + 3TC	5	12.5%	15.95
5	ABC + 3TC + AZT	2	5%	17.68
6	AZT + 3TC + LPv/rtv	3	7.5%	16.94

Table 3: Blood chemistry analysis

Total participants = 121

GROUPS	ENZYMES	COMPARISON WITH MANUFACTURER' (Randox) NORMAL STANDARD	FREQUENCY	PERCENTAGE
A	ALT	≤12 IU/L Normal	30	75%
		>12 IU/L	10	25%
	AST	≤12 IU/L Normal	10	25%
		>12 IU/L	30	75%
	AST/ALT	≤1 Normal	6	15%
		>1	34	85%
	Male Creatinine	53- 97 µmol/l (Normal)	10	25%
		Outside normal range	9	22.5%
	Female Creatinine	44-80 µmol/l (Normal)	11	27.5%
		Outside Normal range	9	22.5%
	Male Creatinine	97-137ml/min/1.732m^2 (Normal)	3	7.5%

	Clearance	Outside normal range	16	40%
	Female Creatinine clearance	88-128ml/min/1.732m^2 (Normal)	6	15%
		Outside normal range	14	35%
	Total Number of Participants		**40**	
B	ALT	≤12 IU/L	33	73.33%
		>12 IU/L	12	26.67%
	AST	≤12 IU/L	6	13.33%
		>12 IU/L	39	86.67%
	AST/ALT	≤1	5	11.11%
		>1	40	88.89%
	Male Creatinine	53- 97 µmol/l	4	8.89%
		Outside normal range	14	31.11%
	Female Creatinine	44-80 µmol/l	13	28.89%
		Outside normal range	14	31.11%
	Male Creatinine Clearance	97-137ml/min/1.732m^2	3	6.67%
		Outside normal range	14	31.11%
	Female Creatinine Clearance	88-128ml/min/1.732m^2	2	4.44%
		Outside normal range	26	57.78%
	Total Number of Participants		**45**	
C	ALT	≤12 IU/L	2	100%
		>12 IU/L	0	0%
	AST	≤12 IU/L	1	50%
		>12 IU/L	1	50%
	AST/ALT	≤1	1	50%
		>1	1	50%
	Male Creatinine	53- 97 µmol/l	1	50%
		Outside normal range	1	50%
	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	2	100%
	Total number of Participants		**2**	
D	ALT	≤12 IU/L	30	88.24%
		>12 IU/L	4	11.76%
	AST	≤12 IU/L	20	58.82%
		>12 IU/L	14	41.18%
	AST/ALT	≤1	5	14.71%
		>1	29	85.29%
	Male Creatinine	53- 97 µmol/l	11	32.35%
		Outside normal range	5	14.70%
	Female Creatinine	44-80 µmol/l	10	29.41%
		Outside normal range	7	20.59%
	Male Creatinine Clearance	97-137ml/min/1.732m^2	1	2.94%
		Outside normal range	11	32.35%
	Female Creatinine clearance	88-128ml/min/1.732m^2	3	8.82%
			10	29.41%
	Total number of Participants		**34**	

Table 4: EFFECT OF HAART ON LIVER AND KIDNEY

GROUPS	**Liver function test (mean±SD)**		**Kidney function test (mean±SD)**	
	ALT (IU/L)	**AST (IU/L)**	**Cr (µmol/L)**	**Cr clearance (ml/min/1.732m^2)**
A (n=40)	9.90±5.40	16.84±8.71	54.22±22.38	117.91±115.37
B (n= 45)	9.99±5.87	23.55±12.41	44.22±19.41	74.64±57.80
C (n=2)	6.32±0.79	8.54±10.09	52.44±35.75	71.15
D (n=34)	5.20±3.64	11.23±6.07	59.18±23.71	82.58±35.92

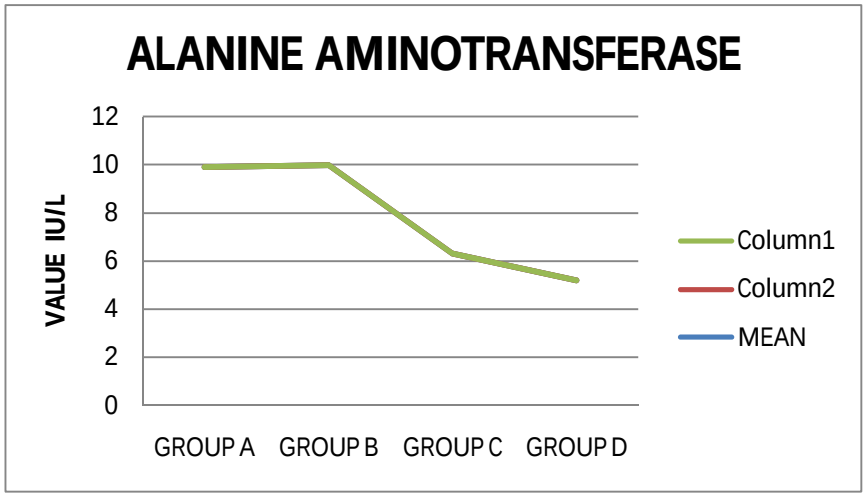

Figure 1: Comparison of Mean Value of Alanine Aminotransferase Among Groups

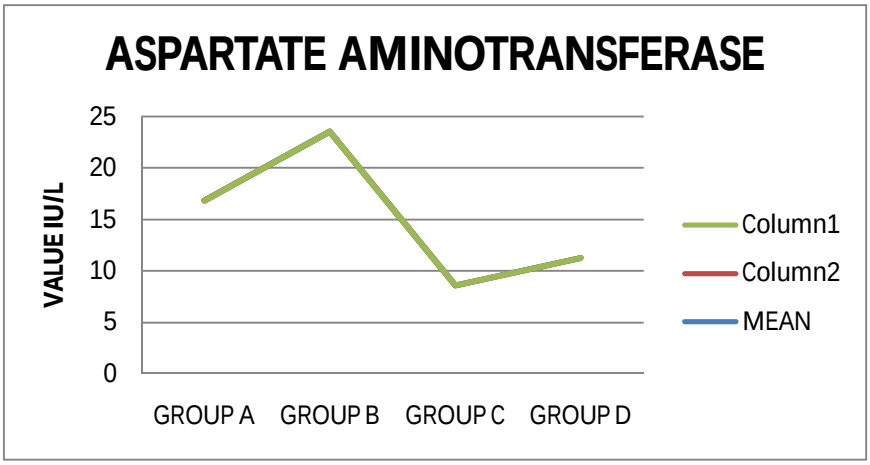

Figure 2: Comparison of Mean Value of Aspartate Aminotransferase Among Groups

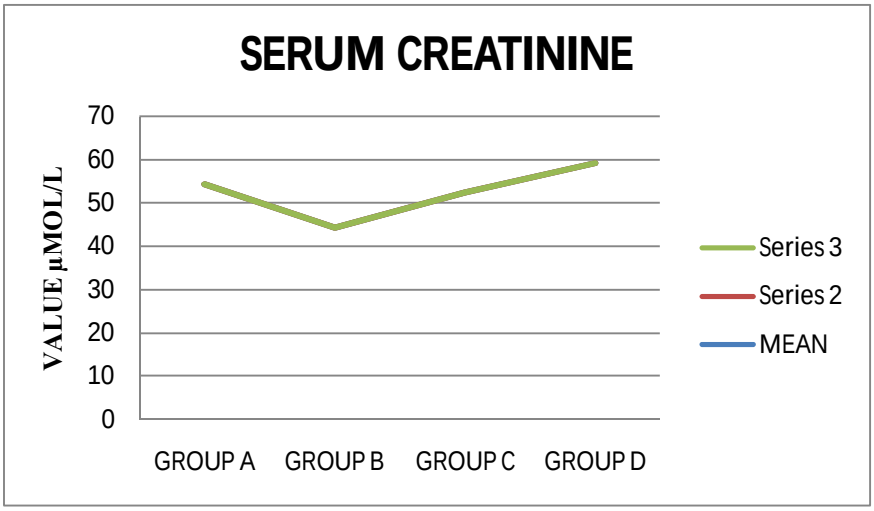

Figure 3: Comparison of Mean Value of Serum Creatinine Among Groups

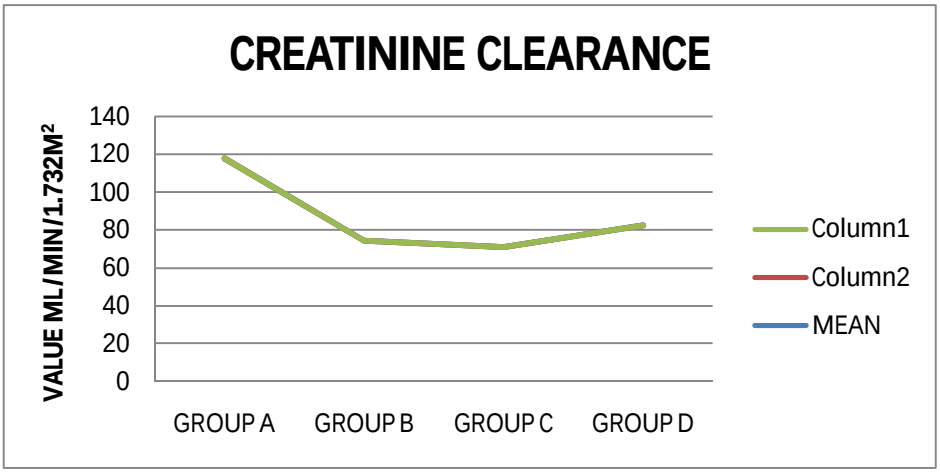

Figure 4: Comparison of Mean Value of Creatinine Clearance Among Groups

DISCUSSION

The BMI of 0-5 years children in Group A was less than that of the children in group B, C and D which implies that HAART reduced BMI of the children who took the various HAART combinations in this study. Only children that took ABC + 3TC + AZT had BMI greater than those of the control children, while other HAART combinations were responsible for the BMI reduction. Greater than half of the study participants were on AZT+ 3TC+ NVP combination because it was well tolerated.

Liver enzyme ALT was elevated in a quarter of the children taking HAART combination while three quarter of them had elevated liver enzyme AST. This implies that liver enzyme AST was more affected by the HAART. Almost all the children receiving HAART had AST/ALT ratio greater than 1.0 which confirmed presence of liver injury. This study has supported claims made by previous researchers (Ugiagbe et al., 2012, Nunez M, 2010).Slightly more than half (52.5%) of the study participants who took HAART combination had normal creatinine while almost half of them (45%) had elevated creatinine which indicated potential damage to kidney. The creatinine clearance of the study participants who took HAART combination showed that only 22.5% of the participants in this group had normal creatinine clearance while three quarter (75%) had abnormal creatinine clearance, an indication that the kidney function had already been impaired (Madeddu et al 2006, Rho et al., 2007) .

Among study participants who took nevirapine for six weeks and later continued with co-trimoxazole, less than thirty percent of them had elevated liver enzyme ALT and more than

eighty percent had elevated liver enzyme AST. Greater than eighty-five percent of the study participants in this group had AST/ALT ratio greater than 1.0. Since all the study participants in this group had taken nevirapine, one of the drug combination used in HAART which also affect liver enzymes. The effect of nevirapine on the liver of these children may either be a permanent damage or may be resolved much later in the future. The kidney function test among these study participants showed that less than forty percent of them had normal creatinine while greater than sixty percent had abnormal creatinine. This implied that there would be potential damage for the kidney in future unless the effect of nevirapine was resolved after nevirapine was stopped. About eighty-eight percent of the participants on nevirapine and co-trimoxazole had abnormal creatinine clearance. This is related to the use of nevirapine which was taken for six week and possibly implied a permanent impairment of their kidney function.

There were only two volunteers of HIV-infected children who received only co-trimoxazole, the two had contrasting result of AST, AST/ALT and creatinine except ALT and creatinine clearance where they both had normal and abnormal values respectively. Hence, it would not be used for comparison with other groups.

The control children did not did not receive any HAART drugs nor infected with HIV, however more than half of them had elevated AST and AST/ALT ratio. Thirty-five percent of the control children had abnormal creatinine and sixty percent had abnormal creatinine clearance. Other medications received from the clinic before enrollment of the control children for the study could have affected the result.

The mean value of liver enzymes ALT and AST was highest among children who took nevirapine and co-trimoxazole and HAART combination drugs. This clearly showed that nevirapine and other HAART drugs affect liver enzymes ALT and AST. Though the mean value of ALT was normal but AST was abnormal for the children who took nevirapine and co-trimoxazole and those who took the HAART combination drugs. The implication of the elevated liver enzymes AST suggests presence of liver injury. This result is supported previous researchers (Ugiagbe et al., 2012, Nunez M, 2010). The mean value of serum creatinine was normal for children who took HAART combination drugs, nevirapine and co-trimoxazole, cotrimoxazole only and the control children. But the mean value of serum creatinine was slightly higher for the children who took HAART combination and the control children. The mean value of creatinine clearance was highest for the children who received

HAART combination drugs and the only value in the normal range. The mean value of creatinine clearance for the control children was slightly less than the normal range. This showed that HAART combination drugs had helped to improve the kidney function. This affirmed earlier claims made by previous studies (Madeddu et al 2006, Rho et al., 2007)

The statistical analysis showed that there was significant effect at 99% confidence interval that liver enzymes ALT and AST for children on HAART were greater than other children that participated in study. Similarly at 90% confidence interval, there was significant effect that both serum creatinine and creatinine clearance of children receiving HAART were greater than other children that participated in the study. The statistical result confirmed the association of liver injury, immediate improvement of kidney function with possibility of future kidney malfunction with the use of highly active antiretroviral therapy among children below five years old.

CONCLUSION

This study has shown that HAART combination drugs are responsible for liver injury as well as improved kidney function with possibility of future kidney malfunction in children of zero to five years old.

RECOMMENDATION

A regular monitoring of liver enzymes in children receiving HAART combination drugs is very important to prevent permanent liver damage.

ABBREVIATION

ALT: Alanine aminotransferase
AST: Aspartate aminotransferase
HAART: Highly Active Antiretroviral Therapy
HIV: Human immunodeficiency virus
ART: Antiretroviral therapy
AIDS: Acquired immunodeficiency syndrome
ABC: Abacavir
3TC: Lamivudine
LPv: Lopinavir
D4T: Stavudine
NVP: Nevirapine
AZT: Zidovudine

EFV: Efavirenz

Rtv: Ritonavir

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