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Impact of Highly Active Antiretroviral Therapy (HAART) on Organs of HIV Infected Children in Abia State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author MOA designed the study, wrote protocol, collected data, performed the statistical analysis and wrote the first draft of the manuscript. Authors MKO and JOM participated in study design and supervised work. Author JOM managed the administrative process. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: The aim of the study is to detect effect of zidovudine + lamivudine+nevirapine (AZT+3TC+NVP) on liver and kidney.
Study Design: Sixty-four children age 0 to 5 years attending FMC Umuahia were enrolled for the study in four groups.
Place of Study: Study was carried out in Antiretroviral Clinic and Pediatrics Clinic of Federal Medical Center, Umuahia in Abia State, Nigeria from February 2012 to December 2012.
Methodology: Fourteen asymptomatic immunocompromised HIV-infected children receiving HAART, AZT+3TC+NVP were in Group A. Thirty-five HIV-exposed children who initially received nevirapine for six weeks and later continued with co-trimoxazole were in Group B. Eight non-immunocompromised HIV-infected children who only received co-trimoxazole were in Group C. Group D had seven normal HIV-seronegative children who served as control for the study. A 2 ml blood was collected from the children, centrifuged to obtain serum which was analyzed in the laboratory for liver function test (AST, ALT) and kidney function test (creatinine, creatinine clearance).

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Result: Thirty-three (51.6%) of the participants were male while thirty-one (48.4%) were female. ALT was highest in Group A (14.69 IU/L) followed by Group B (13.54 IU/L), Group D (7.77 IU/L) and Group C (6.12 IU/L) respectively. AST was highest in Group A (39.42 IU/L), followed by Group B (32.22 IU/L), Group C (18.66 IU/L) and Group D (14.46 IU/L) respectively. Creatinine was highest among children in Group A (57.17 µmol/l) followed by Group C (56.49 µmol/l), Group B (53.06 µmol/l) and Group D (35.42 µmol/l). Creatinine clearance was highest in Group A (100.58 ml/min/1.732m^2), followed by Group D (84.48 ml/min/1.732m^2), Group C (74.69 ml/min/1.732m^2) and Group B (61.17 ml/min/1.732m^2) respectively. The BMI was highest in Group A (32.89 kg/m^2), followed by Group B (26.33 kg/m^2), Group C (22.03 kg/m^2) and Group D (17.34 kg/m^2) respectively.
Conclusion: The study showed that HAART has significant effect on elevation of BMI and serum creatinine.

Keywords: HIV; HAART; antiretrovirals; liver; kidney; children.

1. INTRODUCTION

In 2010, it was reported that there were 34 million people living with HIV in the world with 2.7million people newly infected in the same year. 1.8 million People died from AIDS related causes in 2010. Only 6.65 million people received antiretroviral therapy out of which 456,000 were children [1].

In 21st century, International community faced formidable health and developmental challenges in countries in the poorest regions. HIV epidemic has already dramatically reversed decades of progress on key development indicators like infant mortality and life expectancy [2]. AIDS has been regarded as one of the causes of adult deaths in sub-Saharan Africa and the full scourge of the epidemic was felt in 2006 as more than 2.2 million people died from AIDS related causes [3].

There has been remarkable progress in reducing the HIV incidence among children younger than 15 years in sub-Saharan Africa. The estimated 350,000 children who were newly infected with HIV in 2010 in sub-Saharan Africa were 30% fewer than the 500,000 who acquired HIV infection in 2001. Fewer children are dying from AIDS related causes, from an estimated 320,000 in 2005 to 230,000 in 2010 [4].

Mother- to –child- transmission (MTCT) of HIV was estimated to result in greater than 90% of infections worldwide in infants and children, it occurred probably late in pregnancy or during birth [5].

Although HAART has been shown to improve renal function, long term use may be associated with significant nephrotoxicity, especially tenofovir and releated nucleotide analogs [6,7]. Also, hepatic steatosis and abnormal liver function tests are common side effects of several antiretrovirals [8,9].

Therefore, this study shall evaluate the impact of HAART on these target organs in HIV-infected children.

2. METHODS

There were sixty-four (64) children aged 0 to 5 years who participated in this research in Southeastern Nigeria after ethical approval was received from Federal Medical Center, Umuahia to start the study. The study started in Antiretroviral Therapy Clinic and later continued in Children Outpatient Clinic of the Hospital. Parents/ Guardians of HIV infected children, HIV-exposed children and normal children were informed of the study. Consents were sought from the care-givers that is parents and guardians. The accepted care givers were given Informed consent to fill. A one-on-one discussion was held with each care giver. The recruited children, both male and female were assigned to four groups. Group A consisted of asymptomatic HIV infected children in Stage 1 who had started Highly Active Antiretroviral Therapy (HAART) for at least three months. All the participants in this Group were on HAART combination, NVP+3TC+AZT. Group B 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 HIV- seronegative. Groups C consisted of confirmed HIV-infected children with higher CD4 count and were not eligible to start HAART at age above 2 years. Group D 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. A 2 ml blood was collected from each of the recruited children. The blood samples were taken to the laboratory for blood chemistry analysis. The blood samples were spun 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.

3. LABORATORY EVALUATION

3.1 Alanine Aminotransferase (ALT) (Randox) IU/L

Using measurement against sample blank:

Pipette was used to add 0.5 ml of buffer into a test tube without serum as the sample blank. Pipette was used to add 0.5 ml of buffer and 0.1 ml of serum into another test tube known as the sample. Each of the test tubes was mixed and incubated for exactly 30 minutes at 37ºC. Pipette was used to add 0.5 ml of 2, 4-dinitrophenylhydrazine and 0.1 ml of sample into the sample blank tube. 0.5 ml 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 20 minutes at 25ºC. Then, 5.0 ml 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.

Same procedure was repeated for AST (IU/L) and Creatinine (µmol/l). 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 leaflets. Creatinine clearance was derived from serum creatinine by using Schwartz equation and Shull equation.

3.2 Schwartz Equation

CrCl (ml/min/1.73m2)=[length (cm) x k] / Scr [10], K=0.45 for infants 1 to 52 weeks old, K=0.55 for children 1 to 13 years old.

3.3 Shull Equation

Crcl (ml/min/1.73m2) = ((0.035 x age) + 0.236) x 100)/ Scr [11]. (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.

4. RESULTS

Thirty-three (51.6%) of the participants were male while thirty-one (48.4%) were female. The mean age of the study participants was 26.56 months and their average weight was 15.57 kg. The average Body Mass Index (BMI) for these 0-5 years study participants in their respective groups was as follows, Group A had highest value of BMI (32.89 kg/m^2) followed by Group B (26.33 kg/m^2), Group C (22.03 kg/m^2) and Group D (17.34 kg/m^2) respectively Table 1.

Table 1. Basic Characteristics of Participants

Characterstics	N= 64
Male	33 (51.6%)
Female	31 (48.4%)
Mean age (months)	26.56±24.27
Average Weight (Kg)	15.57±9.87
BMI (A)	32.89±21.87
BMI (B)	26.33±9.64
BMI (C)	22.03±8.13
BMI (D)	17.34±2.84
Mean baseline CD4 cells/mm^3	1189.5±1143.3
WHO Stage	1

The result of participants in Group A showed that nine (64.3%) of them had normal result for ALT while five (35.7%) had elevated ALT. Group A also showed that four (28.6%) of the group participants had normal AST while ten (71.4%) had elevated AST. There were two (14.3%) children with normal AST/ALT ratio and twelve (85.7%) children with elevated AST/ALT ratio. Only one (7.1%) male participants had normal creatinine while four (28.6%) male had abnormal creatinine values. Five (35.7%) female participants had normal creatinine values and four (28.6%) female participants had abnormal creatinine Table 2.

In Group B, twenty-one (60%) participants had normal ALT while fourteen (40%) had elevated ALT. Five (14.3%) of the research participants had normal AST while thirty (85.7%) had elevated ALT. Two (5.7%) participants had normal AST/ALT ratio while thirty-three (94.3%) had elevated AST/ALT ratio. Among the research participants in Group B, eight (22.9%) male participants had normal creatinine while thirteen (37.1%) had abnormal creatinine. Eight (22.9%) female participants had normal creatinine and five (14.3%) female participants had abnormal creatinine Table 2.

In Group C, seven (87.5%) of the participants in this group had normal ALT. One (12.5%) of the participants had normal values of AST while seven (87.5%) had elevated values. One (12.5%) male participants had normal creatinine. Three (37.5%) female participants had normal values of creatinine while four (50%) had abnormal creatinine Table 2.

Table 2. Comparison of blood chemistry results with normal standard values

Groups	Enzymes	Comparison with manufacturer'normal standard (Randox[14,15,16])	Frequency	Percentage
A	ALT	≤12 IU/L	9	64.3%
		>12 IU/L	5	35.7%
	AST	≤12 IU/L	4	28.6%
		>12 IU/L	10	71.4%
	AST/ALT	≤1	2	14.3%
		>1	12	85.7%
	Male	53- 97 µmol/l	1	7.1%
	Creatinine	Outside normal range	4	28.6%
	Female	44-80 µmol/l	5	35.7%
	Creatinine	Outside Normal range	4	28.6%
	Male	97-137 ml/min/1.732m^2	2	11.76%
	Clearance	Outside normal range	5	29.41%
	Female	88-128 ml/min/1.732m^2	1	5.88%
	Clearance	Outside normal range	9	52.94%
	Total Number of Participants		**14**	
B	ALT	≤12 IU/L	21	60%
		>12 IU/L	14	40%
	AST	≤12 IU/L	5	14.3%
		>12 IU/L	30	85.7%
	AST/ALT	≤1	2	5.7%
		>1	33	94.3%
	Male	53- 97 µmol/l	8	22.9%
	Creatinine	Outside normal range	13	37.1%
	Female	44-80 µmol/l	8	22.9%
	Creatinine	Outside normal range	5	14.3%
	Male	97-137 ml/min/1.732m^2	0	0%
	Clearance	Outside normal range	21	60%
	Female	88-128 ml/min/1.732m^2	2	5.71%
	Clearance	Outside normal range	11	31.43%
	Total Number of Participants		**35**	
C	ALT	≤12 IU/L	7	87.5%
		>12 IU/L	0	0%
	AST	≤12 IU/L	1	12.5%
		>12 IU/L	7	87.5%
	AST/ALT	≤1	0	0%
		>1	7	87.5%
	Male	53- 97 µmol/l	1	12.5%
	Creatinine	Outside normal range	0	0%
	Female	44-80 µmol/l	3	37.5%
	Creatinine	Outside normal range	4	50%
	Male	97-137 ml/min/1.732m^2	0	0%
	Clearance	Outside normal range	1	12.5%
	Female	88-128 ml/min/1.732m^2	2	25%
	clearance	Outside normal range	5	62.5%
	Total number of Participants		**8**	

Table 2 Continued ……

D	ALT	≤12 IU/L	7	100%
		>12 IU/L	0	0%
	AST	≤12 IU/L	1	14.3%
		>12 IU/L	6	85.7%
	AST/ALT	≤ 1	1	14.3%
		>1	6	85.7%
	Male	53- 97 µmol/l	1	14.3%
	Creatinine	Outside normal range	4	57.1%
	Female	44-80 µmol/l	1	14.3%
	Creatinine	Outside normal range	1	14.3%
	Male	97-137 ml/min/1.732m^2	0	0%
	Clearance	Outside normal range	3	42.86%
	Female	88-128 ml/min/1.732m^2	0	0%
	Clearance	Outside normal range	2	28.57%
	Total number of Participants		**7**	

In Group D, seven (100%) of the participants had normal ALT. One (14.3%) participant had normal AST while six (85.7%) had elevated AST. One (14.3%) participants had normal AST/ALT ratio while six (85.7%) had elevated AST/ALT ratio. One (14.3%) male participant had normal creatinine value and four (57.1%) male participants had abnormal creatinine. One (14.3%) female participant had normal creatinine and another one (14.3%) female participant had abnormal creatinine Table 2.

The liver function test showed that the Group mean of liver enzyme ALT was highest for 0-5 years children in Group A (14.69 IU/L) followed by Group B (13.54 IU/L), Group D (7.77 IU/L) and least for Group C (6.12 IU/L). The mean of liver enzyme AST was highest among children in Group A (39.42 IU/L) followed by Group B (32.22 IU/L), Group C (18.66 IU/L) and Group D (14.46 IU/L) respectively. The kidney function test showed that the mean of creatinine was highest among children in Group A (57.17 µmol/l) followed by Group C (56.49 µmol/l), Group B (53.06 µmol/l) and least for Group D (35.42 µmol/l). The mean of creatinine clearance among the children was highest in Group A (100.58 ml/min/1.732m^2) followed by Group D (84.48 ml/min/1.732m^2), Group C (74.69 ml/min/1.732m^2) and Group B (61.17 ml/min/1.732m^2) respectively Table 3.

Table 3. Impact of HAART on liver and kidney of study participants

Groups	Liver function test (mean±SD)		Kidney function tests (mean±SD)	
	ALT (IU/L)	**AST (IU/L)**	**Cr (µMol/L)**	**Cr clearance (Ml/min/1.732m^2)**
A (n =14)	14.69±22.19	39.42±60.23	57.17±28.73	100.58±51.24
B (n = 35)	13.54±16.12	32.22±40.72	53.06±21.56	61.17±25.95
C (n =8)	6.12±2.83	18.66±6.73	56.49±23.33	74.69±20.57
D (n = 7)	7.77±2.16	14.46±6.68	35.42±36.09	84.48±104.23

ALT (p>0.05), AST (p>0.05), Cr (p>0.05), Cr CI (p>0.05).Dunnett posthoc test showed that creatinine of children in group A was significantly greater than children in group D (p=0.04). It also showed that BMI of children in group A was significantly greater than children in group D (p=0.029)

Fig. 1. Showed that the mean of ALT of participants in Group A was highest followed by Group B, Group D and least for Group C. Fig. 2. Showed that the mean of liver enzyme AST was highest for participants in Group A followed by Group B, Group C and least for Group D. Fig. 3. Showed that the mean of serum creatinine was highest for participants in Group A followed by Group C, Group B and least for Group D. Fig. 4. Showed that the mean of

creatinine clearance was normal for participants in Group D and Group A and below normal for participants in Group B and Group C.

The statistical analysis of variance (ANOVA) using IBM SPSS version 20 showed the p-values as, ALT (p=0.504), AST (p=0.557), serum creatinine (p=0.201) and creatinine clearance (p=0.099). Dunnett posthoc test showed that creatinine of children in group A is significantly greater than children in group D (p=0.04). It also showed that BMI of children in group A is significantly greater than children in group D (p=0.029).

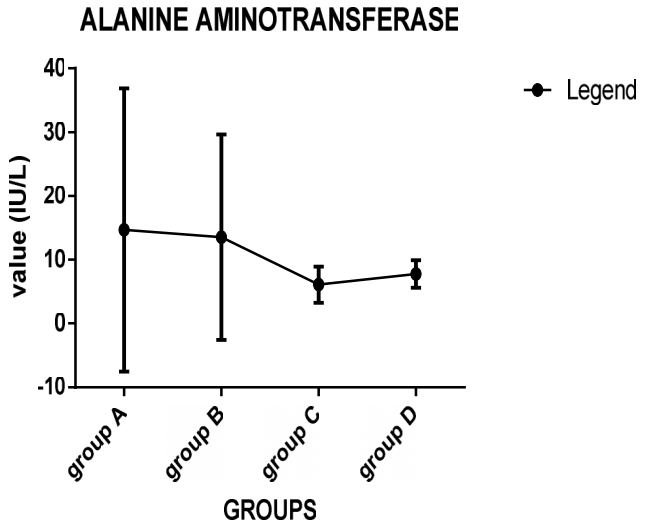

Fig. 1. Comparison of ALT among the groups

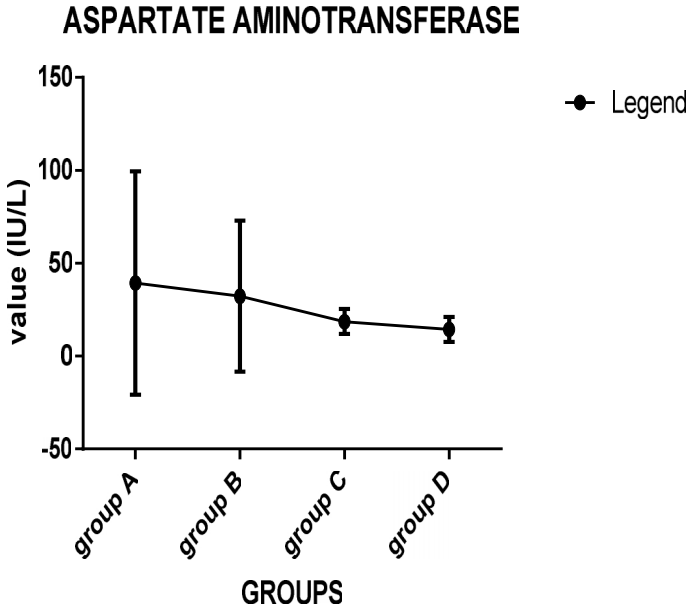

Fig. 2. Comparison of AST among groups

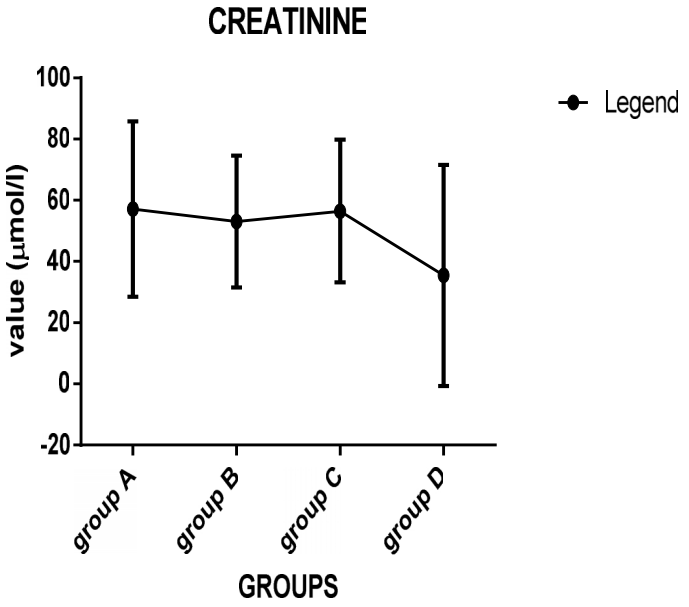

Fig. 3. Comparison of serum creatinine among groups

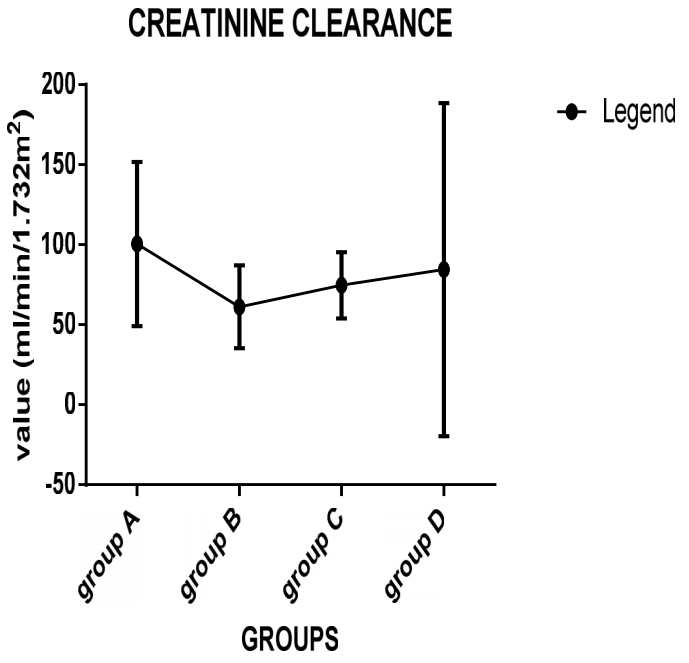

Fig. 4. Comparison of creatinine clearance among groups

5. DISCUSSION

More of the 0-5 years study participants with elevated ALT, AST and AST/ALT ratio were found in groups (A, B and C) except the control group (D). Similarly, high elevation of liver enzymes, ALT and AST was found among children who took HAART regimen and those who took nevirapine for six weeks before continuing on co-trimoxazole. Elevated ALT and AST enzymes are associated with liver injury. This confirmed the report of the study conducted by Ugiagbe et al. [8].

The kidney function test showed that the 0-5 years children who had taken HAART combination, single nevirapine therapy and co-trimoxazole had wide margin of serum creatinine over the control children. This showed the impact of these drugs on the kidney function of children. The group mean of creatinine clearance of the participants on HAART (AZT + 3TC + NVP) and the control group had values that were within the normal range while the other two groups had values below normal creatinine clearance values. Hence, it showed that there was an improved kidney function among children on HAART over the other groups. This claim is in support with earlier studies [6,7]. The HIV-exposed children who took nevirapine and co-trimoxazole had reduced renal function. This was claimed in earlier study by Fontes et al. [12]. This did not imply that there would not be adverse effect of the use HAART on kidney function after a long time of use.

Elevated BMI was also shown to be the effect of intake of HAART regimen and single nevirapine therapy among these children.

Graphical illustration of the group mean of liver enzyme, ALT indicated that the elevation of the enzyme was prominent among children taking AZT + 3TC + NVP and the HIV-exposed children who took nevirapine for six weeks and then continued with co-trimoxazole thereafter. Also, liver enzyme, AST elevation was prominent among HIV infected children on AZT + 3TC + NVP and HIV-exposed children who had nevirapine for six weeks and later continued with co-trimoxazole. The result supported earlier study conducted by Wagner [13]. In previous studies, normal values for ALT, AST and creatinine were established [14,15,16].

Graphical illustration of the group mean for serum creatinine indicated elevation of creatinine among HIV infected children on AZT + 3TC + NVP, HIV-exposed children who took nevirapine for six weeks and continued with co-trimoxazole as well as HIV-infected children with uncompromised immunity who were not taking HAART. The mean of creatinine clearance as shown on the graph illustrated that only the children in the control group and HIV-infected children on HAART had creatinine clearance on the normal line while others fell below the normal line.

The statistical analysis of variance failed to show any reliable effect of AZT + 3TC + NVP on liver enzymes ALT and AST but showed a significant effect of AZT + 3TC + NVP on creatinine and BMI of HIV–infected children over the control children.

6. CONCLUSION

In conclusion, this study showed that the use of HAART regimen among children below five years was associated with significant elevation of BMI and serum creatinine.

7. RECOMMENDATION

Continuous Drug Therapeutic Monitoring is hereby recommended to prevent kidney malfunction and to prevent obesity among children below five years who take HAART regimen.

CONSENT

Consent of participants was obtained before enrolment into the study.

ETHICAL APPROVAL

Ethical approval was granted by the Ethical committee of the Medical Center before commencement of the study.

ACKNOWLEDGMENT

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COMPETING INTEREST

Authors have declared that no competing interests exist.

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APPENDIX

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 scale
- Tape rule
- Methylated spirit
- Cotton wool
- Refrigerator

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=425&id=14&aid=3629