International Journal of Medical, Medicine and Health Sciences
ISSN: 2517-9969
Vol:9, No:5, 2015

Effect of Zidovudine on Hematological and Virologic Parameters among Female Sex Workers Receiving Antiretroviral Therapy (ART) in North – Western Nigeria

N. M. Sani, E. D. Jatau, O. S. Olonitola, M. Y. Gwarzo, P. Moodley, N. S. Mujahid

***Abstract*—**Hemoglobin (HB) indicates anemia level and by extension may reflect the nutritional level and perhaps the immunity of an individual. Some antiretroviral drugs like Zidovudine are known to cause anemia in people living with HIV/AIDS (PLWHA). A cross sectional study using demographic data and blood specimen from 218 female commercial sex workers attending antiretroviral therapy (ART) clinics was conducted between December, 2009 and July, 2011 to assess the effect of zidovudine on hematologic, and RNA viral load of female sex workers receiving antiretroviral treatment in north western Nigeria. Anemia is a common and serious complication of both HIV infection and its treatment. In the setting of HIV infection, anemia has been associated with decreased quality of life, functional status, and survival. Antiretroviral therapy, particularly the highly active antiretroviral therapy (HAART), has been associated with a decrease in the incidence and severity of anemia in HIV-infected patients who have received a HAART regimen for at least 1 year. In this study, result has shown that of the 218 patients, 26 with hemoglobin count between 5.1 – 10g/dl were observed to have the highest viral load count of 300,000 – 350,000copies/ml. It was also observed that most patients (190) with HB of 10.1 – 15.0g/dl had viral load count of 200,000 – 250,000 copies /ml. An inverse relationship therefore exists i.e. the lower the hemoglobin level, the higher the viral load count even though the test statistics did not show any significance between the two (P = 0.206). This shows that multivariate logistic regression analysis demonstrated that anemia was associated with a CD4 + cell count below 50/μL, female sex workers with a viral load above 100,000 copies/mL, who use zidovudine.

Severe anemia was less prevalent in this study population than in historical comparators; however, mild to moderate anemia rates remain high. The study therefore recommends that hematological and virologic parameters be monitored closely in patients receiving first line ART regimen.

***Keywords*—**Female sex worker, Zidovudine, Hemoglobin, Anemia.

N. M. Sani is with the Department of Microbiology, Federal University Dutse, Jigawa State – Nigeria (Corresponding Author: e-mail nuramuhammadsani@gmail.com).

E. D. Jatau and O. S. Olonitola are with the Department of Microbiology, Ahmadu Bello University, Zaria.

M. Y. Gwarzo is with the Department of Chemical Pathology Bayero University, Kano - Nigeria,

P. Moodley is with the Department of Virology, National Health Laboratory Service, University of Kwazulu Natal, Durban/ZA.

N. S. Mujahid is with the Department of Microbiology, Federal University Dutse, Jigawa State – Nigeria.

I. Introduction

HUMAN Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) remains a major public health challenge across the globe. Over 25 million people died of AIDS related illness at the end of 2005 [1]. Africa is the epicenter of this pandemic, yet ironically the region is least able to offer significant intervention strategies to the devastation caused by the virus. With a few exceptions, strategies to prevent the spread of HIV have been relatively unsuccessful, spelling doom for the individual and leading to transmission of resistant virus [2], [3]. Nigeria is currently experiencing an HIV/AIDS epidemic that is estimated to have prevalence of 4.1% in adult populations [4].

Female Sex Workers are major reservoirs of HIV and STI infections in the society [5]. This group of highly mobile population suffers from societal stigma and neglect from Government and other intervention programs. Antiretroviral drugs have been remarkably successful in suppressing HIV-1 infection; however, transmitted drug resistance can reduce the efficacy of first – line regimens. This however, calls for an increasing research interest in prevalence of HIV and immunologic response to antiretroviral treatment among female sex workers in North – Western Nigeria.

Anemia is a common and serious complication of both HIV infection and its treatment. In the setting of HIV infection, anemia has been associated with decreased quality of life, functional status, and survival.

Antiretroviral therapy, particularly the highly active antiretroviral therapy (HAART), has been associated with a decrease in the incidence and severity of anemia in HIV-infected patients who have received a HAART regimen for at least 1 year [6].

II. Materials and Methods

A cross-sectional study was carried out using demographic data and blood specimen from female commercial sex workers attending antiretroviral (ART) clinics in North Western Nigeria. Four (4) out of the seven (7) states were selected based on their HIV prevalence [7]. This group of highly mobile population was found to be attending various ART clinics for their care and treatment services including adherence counseling. Five Milliliters of Venous blood was collected from each client and analyzed for hematology and

viral load quantification respectively.

A. Hematology for Hemoglobin and Lymphocyte Count

Sysmex Hematology counter (KX21N) was used for the evaluation of patients' hemoglobin (Hb) Lymphocytes (total, absolute and percentage) as well as packed cell volume according to manufacturer's guide.

B. HIV– 1 RNA Quantification (Viral Load Assay)

The stored plasma samples were packaged and transported to Department of Virology, National Health Laboratory Services, Albert Ikonsi Luthuli Central Hospital, Kuazulu Natal, Durban – South Africa for viral load quantification. The shipment was successfully done in accordance with international standards for biological products through FEDEX Courier services. Abbott Real Time HIV -1 m2000sp model was used for RNA extraction and amplification of Patients' sample.

III. Principles of the Test

The Abbott Real – Time HIV – 1 assay uses Polymerase Chain Reaction (PCR) technology with homogenous real – time fluorescent detection. Partially double – stranded fluorescent probe design allows detection of diverse group M subtypes and group O isolates.

The assay was standardized against a viral standard from the virology quality assurance (VQA) Laboratory of the AIDS Clinical Trial Group [8] and against World Health Organization [9] 1st international Standard for HIV – 1 RNA (97/656) and the assay results were reported in copies/ml [10-11]. The Abbott Real –Time HIV – 1 assay uses TR – PCR [12], to generate amplified product from the RNA genome of HIV – 1 in clinical specimens. An RNA sequence that is unrelated to the HIV – 1 target sequence was introduced in to each specimen at the beginning of the sample preparation. This unrelated RNA sequence is simultaneously amplified by RT – PCR, and serves as an internal control (IC) to demonstrate that the process has proceeded correctly for each sample.

The amount of HIV – 1 target sequence that was present at each amplification cycle was measured through the use of fluorescent – labeled oligonucleotide probes on the Abbott *m*2000*rt* instrument.

The probes do not generate signal unless they are specifically bound to the amplified product. The amplification cycle at which fluorescent signal was detected by the Abbott *m*2000*rt* is proportional to the log of the HIV – 1 RNA concentration present in the original sample.

IV. Sample Preparation

The purpose of sample preparation was to extract and concentrate the target RNA molecules to make the target accessible for amplification and to remove potential inhibitors of amplification from the extract. The Abbott *m*2000*sp* sample preparation system (4x24 preps) uses magnetic particle technology to capture nucleic acids and washes the particles to remove unbound sample components. The unbound nucleic acids are eluted and transferred to output tubes or a deep- well plate. The nucleic acids are then ready for amplification.

The internal control (IC) was taken through the entire sample preparation procedure along with the calibrators, controls and specimen. The stored plasma samples were brought to and thawed at room temperature (15 – 30^{o}C) and later centrifuged at 6,000 revolutions /min. After removing debris and fat from the samples, the plasma samples were transferred to 5ml test tubes before loading on to the machine (Abbott *m*2000*sp*). The test tubes were placed in a rack with controls (negative, low positive and high positive) to make up 96 (93 samples plus 3 controls).

V. Reagent Preparation and Reaction Plate Assembly

The Abbott m2000*sp* combines the Abbott Real Time HIV-1 amplification reagent components (HIV – 1 Oligonucleotide reagent, Thermo stable rTth polymerase enzyme and activation Reagent). The Abbott *m*2000*sp* dispenses the resulting master mix to Abbott 96 –well optical reaction plate along with aliquots of the nucleic acid samples prepared by the Abbott *m*2000 *sp*. The plate was made ready after manual application of the optical seal for transfer on to the machine.

VI. RNA Extraction from the Patients' Sample

To 2-clean reaction vessel, 2 bottles each of lysis buffer containing 70mls of 100mM Tris solution containing 4.7 M Guanidiumisocyanate (GITC) and 10% Tween -20 at pH of 7.8 were added and thoroughly mixed. This causes lysis of the virions, membrane disruption, protein denaturation and inactivation of RNAses. To another reaction vessel, 4-bottles each containing 46mls (184mls) of wash solution 1 containing 3.5M Guanidiumisocyanate, 5% Tween 20 and 50Mm potassium oxaloacetate (KOAc) , at a pH 6.0 were added. The last reaction vessel was filled with RNAse free water (wash solution 2). Both wash 1 and 2 removes cellular debris/proteins and salts which are potential inhibitors, hence provides a free RNAse free environment. Two bottles containing 13mls of magnetic particles and four bottles containing 1ml each of elusion buffer were also prepared and air bubbles dispensed.

VII. Extraction Procedure for RNA Quantification (Viral Load)

The machine, Abbott *m*2000*sp* was switched on and allowed to pre-heat and incubate for 5-6 minutes. Magnetic particles and internal controls (IC) were pre-mixed accordingly and distributed to all the 96 clean tubes by dispensing 0.28µl to each tube.

The machines automatically pipettes and dispense the lysis buffer to each of the tubes containing magnetic particles followed by 1ml of the patients' sample and incubated at 50^{0}C for 20 minutes. An iron oxide magnetic particle (rusty color) in basic pH and high concentration, the backbone of the RNA molecule becomes exposed, binding to the magnetic micro particles. This was washed 4 times using wash 1 and 2 solution. Viral RNA was eluted where the machine

automatically added elution buffer containing 20 mm Potassium Phosphate, pH 8.5 at 750C for 20 minutes.

The elution was repeated to allow for competitive removal of nucleic acid from positively charged Fe+ leaving behind the pure RNA ready for binding with amplification reagents (Probes, Primers, Activators, etc.).

VIII. Nucleic Acid Amplification and Detection

The amplification kits (Activator, Primers, Probes, dNTPs, rTth Polymerase and internal controls) and reagents were thawed at 15 – 300C. Each assay calibrator and control was vortexes three times for 2 – 3 seconds before use. The Abbott *m*sample preparation bottles were gently inverted to ensure a homogeneous solution. A calibrated precision pipette dedicated for internal control was used to add 500µl of IC to each bottle of *m*lysis buffer and content gently mixed by inverting the container 5 – 10 times to minimize forming.

The patients' sample was vortexed three times for 2- 3 seconds and the low and high positive controls, negative controls and calibrators were placed into the Abbott *m* 2000*sp* sample rack. Five milliliter (5ml) reaction vessels were placed in to Abbott *m*2000*sp* 1ml subsystem carrier in addition to loading the Abbott *m*2000*sp* sample preparation system reagent. The Abbott 96 Deep – Well plate on the Abbott *m*2000*sp* work table and appropriate application file was selected on the screen from the protocol that corresponds to 0.6mls of the sample being tested.

The sample extraction protocol was initiated as described in the Abbott *m*2000*sp* operation manual. Gloves were changed at this point before handling the amplification kits. The amplification reagents and master mix vial were loaded on the *m*2000sp work table after completion of sample preparation. The machine was switched on to initialize the Abbott *m*2000 *rt* instrument in the amplification area. Again, gloves were removed at this point before returning to the sample preparation area.

The Abbott 96 Deep – Well optical reaction plate was sealed after the Abbott *m*2000*sp* has completed the addition of samples master mix according to the Abbott *m*2000*sp* operations manual. The sealed Optical reaction plate was carefully placed on the splash free support base for transfer to the Abbott *m*2000*rt* instrument. The Abbott 96 Deep – Well Optical reaction plate was placed in Abbott *m*2000*rt* instrument. Appropriate application file was selected on the screen from the protocol that corresponds to 0.6mls of the sample being tested and Abbott Real time HIV -1 was initiated as described in the *m*2000 *rt* manual.

IX. Statistical Analysis

The data collected was validated using the source documents (Laboratory register, Work sheet, Questionnaire etc.) and subjected to statistical analysis using One –Way ANOVA, Wilcoxin Signed rank test, Pearson's Correlation, Mann Whitney and Chi-Square tests respectively to draw out inferences at 95% confidence intervals. All testing were performed at 5% level of significance using Statistical Package for Social Sciences (SPSS) version 17 respectively.

X. Discussion

Historically, sex workers have been blamed for spreading sexually transmitted diseases including HIV. Determining HIV prevalence among this group of highly mobile population has been quite challenging. Like other marginalized populations, sex workers often receive little attention from both public health officials and researchers. The stigma attached to sex work and criminal sanctions made reliable data hard to come by and there is almost no information about male sex workers [13], [15]. The mean age of sex workers was found to be 31 years. However, their state of sexual activities indicates that 81 (37.2%), 59 (27.1%) and 51 (23.4%) of female sex workers who participated in the study were from Kaduna, Kebbi, and Kano respectively. Similarly, a total of 27 (12.1%) sex workers from Jigawa State were used for the study (Figure 2).

Treatment with first and second line antiretroviral drugs (ARVs) is one of the breakthroughs that successfully prolonged the life of people living with HIV/AIDS (PLWHA) globally. From this study, it was observed that different combinations of ARVs (AZT, 3TC, d4T, Truvada and other combinations) were used by the study population. Table I shows the effect of ART combination and condom use on patients' viral load. It was observed that most patients that use condom were found to be on zidovudine based (AZT, 3TC, NVP) combination and 75 (92.6%) had a viral load count within the range of 0 – 50,000 copies/ml.

Our findings have shown that there was negative or no correlation observed between hematological parameters and ART combination (Table II) $P > 0.005$.

While an inverse relationship exists between HB and viral load however, direct relationship was observed in the case of packed cell volume (PCV) of the studied population as shown in Table III. It is known that the normal PCV ranges from 36 – 46% (Women) and 37 – 49% (Men). From Table III, it was observed that majority of the studied population (121) with PCV of 30.1 – 35.0% had a viral load count of 0 – 250,000. The highest count of 300,000 – 30,000 was observed in 1(9.1%) patient with corresponding PCV value of 20.1 – 30% respectively.

This therefore indicates that the patient's viral load increases with PCV hence, a strong association between packed cell volume and viral load ($P<0.005$).

However, 1(2.0%) patient was found to have a viral load count between 300,000 – 350,000 copies/ml. It was also observed that 35 (94.6%) of the sampled population who use condom were taking stavudine-Lamivudine-Nevirapine (d4T, NVP, 3TC) combination with viral load of 0 – 50,000 copies/ml. However, 2 (100%) of condom users that use other ART combinations (Truvada, Efavirenz and Truvada – Nevirapine) had a viral load of 0 – 50,000 copies/ ml. It was also observed from the table that 1(1.4%) from non- condom users with AZT combination had a viral load of 200,000 – 250,000 copies/ml. Generally speaking, no strong association observed between ART combination and patients viral load

count (Table IV).

In this study, it was found that several antiretroviral drugs mostly of first line category (Zidovudine, Lamivudine, Nevirapine, etc.) were used by the studied population. From this study, Table I has shown that, Zidovudine (AZT) combination was the most commonly used regimen by the patients with 152(69.75%) of the studied population.

The high number of patients on AZT might be due to the fact that most of the clinics use first line antiretroviral (ART) except a few cases on second line regimen due to treatment failure as a result of poor adherence and lost to follow up (LTFU).

The choice of ART is usually based on its availability, the national guideline, clinician's decision, patients response to treatment and clinical/pharmacological history especially the side effects. This agrees with [14] that reported that majority of patients (46%) and 41%, respectively) were initiated on Zidovudine – containing or Stavudine – containing first line regimens. Although the Chi – Square test did not suggest significant association between ART combination and Viral load count (P = 0.942), our findings show that 75 (92.6%) of patients on AZT (Zidovudine, Lamivudine and Nevirapine) combination had a viral load count between 0 - 50,000 copies/ml while 35 (94.6%) of those on d4T (Stavudine, Lamivudine and Nevirapine) combination recorded a viral load count of 0 – 50,000 copies/ml (Table IV).

In both combinations, a viral load count of 150,000 – 300,000 copies/ml was observed in only 1.2% and 2.7% for zidovudine and stavudine combinations respectively. However, the highest viral count of 300,000 – 350,000 copies/ml was observed in 1(1.2%) despite the fact that this patient was on AZT combination for 1 year with no treatment default or missed clinic appointment. This might possibly suggests adherence problem and the likelihood of treatment failure. Generally a low viral count was observed in other combinations (Truvada, Nevirapine, Efavirenz as well as Truvada/EFZ) respectively.

XI. Conclusion

The study's observation on antiretroviral treatment also revealed that most patients are on first line Zidovudine (AZT) and Stavudine (d4T) - based combination, accounting for 69.75% and 26.10% respectively. It was observed that he high percentage use of AZT in the studied population was based on availability of ARVs in the country, patient's response to treatment (prognosis), clinical, immunological as well as pharmacological data including side effects and acute drug reaction (ADR).

In view of the fact that majority of AIDS patients are beginning to fail on first line regimen, coupled with mobile nature of female sex workers, it is imperative to:

1. Sequence viral genome from these patients in order to determine the genetic basis of resistance to treatment especially to zidovudine combination and assess the degree of anemia.
2. Uninterrupted supply of antiretroviral drugs especially the second line regime for the teaming patients by strengthening the supply chain management system
3. Vaccine development effort should be intensified in addition to correct and consistent use of condom to prevent the spread of HIV across the globe.

XII. Results

TABLE I
Clinical Characteristics of Female Sex Workers (N = 218) from North – Western Nigeria

Parameter	No (%)	P- value
ART combination		
Zidovudine (AZT)-based	152 (69.75)	0.942
Stavudine (d4T)-based	57 (26.10)	
Tenofovir (TDF)-based	8 (3.70)	
Lopinavir-based	1 (0.5)	
Haemoglobin†	11.90 (1.6)	0.206
Packed cells volume†	31.40 (3.90)	
Total lymphocytes count*	4.75 ± 1.49	
Absolute lymphocytes count†	1.80 (0.80)	
% lymphocytes*	42.23 ± 10.64	

*Plus-minus values are means ± SD; †Median (IQR)

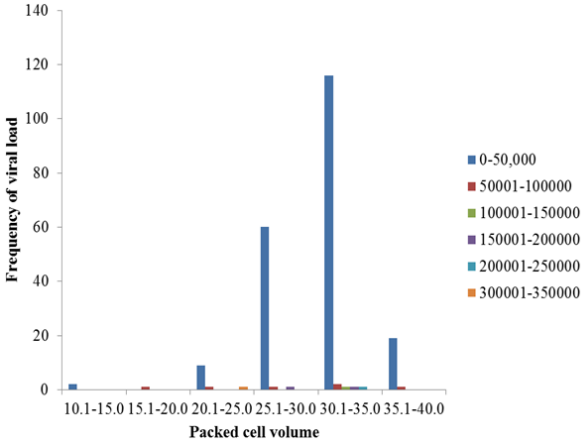

Fig. 1 Packed Cell Volume

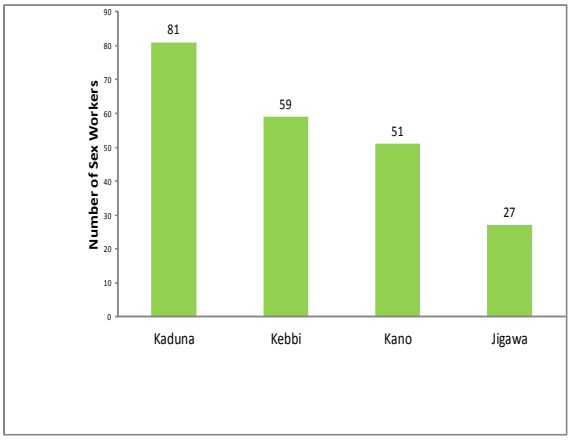

Fig. 2 Distribution of Sex Workers by State

TABLE II
CORRELATION ANALYSIS OF CLIENTS' HEMATOLOGICAL PROFILE WITH SEXUAL AND TREATMENT CHARACTERISTICS (N = 218) FROM NORTH – WESTERN NIGERIA

Parameter	r-value	p-value[a]
No. of sex partners		
Haemoglobin count	0.013	0.844
Total lymphocytes count	0.027	0.688
Absolute lymphocytes count	− 0.045	0.514
ART combination		
Haemoglobin count	0.001	0.992
Totl lymphocytes count	− 0.23	0.735
Absolute lymphocytes count	− 0.037	0.584
Sex trade duration		
Haemoglobin count	0.06	0.380
Total lymphocytes count	0.05	0.437
Absolute lymphocytes count	− 0.088	0.194

[a]Pearson correlation was applied in calculating r and p values.

TABLE III
COMPARISON BETWEEN PATIENTS' PACKED CELL VOLUME (PCV) AND VIRAL LOAD

PCV Range	Viral load Range (Copies / ml)						Total
	0-50,000(%)	50001-100000(%)	100001-150000(%)	150001-200000(%)	200001-250000(%)	300001-350000(%)	
10.1-15.0	2(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	N 0(0.0)	2
15.1-20.0	0(0.0)	1(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1
20.1-25.0	9(81.8)	1(9.1)	0(0.0)	0(0.0)	0(0.0)	1(9.1)	11
25.1-30.0	60(96.8)	1(1.6)	0(0.0)	1(1.6)	0(0.0)	0(0.0)	62
30.1-35.0	116(95.9)	2(1.7)	1(0.8)	1(0.8)	1(0.8)	0(0.0)	121
35.1-40.0	19(95.0)	1(5.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	20
Total	206(94.9)	6(2.8)	1(0.5)	2(0.9)	1(0.5)	1(0.5)	217

$\chi^2 = 59.190$ $p < 0.001$*

TABLE IV
EFFECT OF ART COMBINATION ON PATIENTS' VIRAL LOAD

Condom use	ART Combination	Viral load (copies/ml) N = 218							Total
		0-50000	50001-100000	100001-150000	150001-200000	200001-250000	250001-300000	300001-350000	
Yes[#]	AZT, 3TC, NVP	75(92.6%)	3(3.7%)	1(1.2%)	1(1.2%)	0(0.0%)	0(0.0%)	1(1.2%)	81
	d4T, 3TC, NVP	35(94.6%)	1(2.7%)	0(0.0%)	1(2.7%)	0(0.0%)	0(0.0%)	0(0.0%)	37
	Truvada, Nev/Efar	1(100%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	1
	Truvada, EFZ	2(100%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	2
	Truvada, NVP	2(100%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	2
No[##]	AZT, 3TC, NVP	70(98.6%)	0(0.0%)	0(0.0%)	0(0.0%)	1(1.4%)	0(0.0%)	0(0.0%)	71
	d4T, 3TC, NVP	17(89.5%)	2(10.5%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	19
	Truvada, Nev/Efar	1(100%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	1
	Truvada, NVP	1(100%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	1
	TDF, NVP, 3TC	1(100%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	1
	Lopinvir, Ritonavir, Truvada	1(100%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	1
									217

$\chi 2 = 1.741$ $p = 1.000$; $R = -0.059$ $p = 0.517$ ## $\chi^2 = 8.352$ $p = 0.595$; $R = -0.008$ $p = 0.942$

ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of the Ministries of Health, Brothel and Hotel owners, female sex workers as well as people living with HIV/AIDS (PLWHA) in Kano, Kaduna, Jigawa and Kebbi States, for their support and cooperation.

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