Original Research Article

Biochemical and hematological impact of Hepatitis C Virus (HCV) on Human Immunodeficiency Virus (HIV) infected persons on Antiretroviral Drugs (ARDs) in Nigeria.

Ya'aba Yakubu[1,2]*, Mohammed Shehu Busu[1], Nfrongeh Joseph Fuh[2], Olatunji Kazeem Toyosi[1], Abubakar Aisha[1], Usoroh Mary[1], Daniel Oluwaseun Christiana[1] and Abdulmumin Adam Raji[3]

[1]Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria.

[2]Department of Microbiology, Federal University Lafia, Nasarawa State, Nigeria.

[3]Department of Microbiology, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria.

ABSTRACT

Introduction: Biochemical and hematological abnormalities are among most common clinico-pathological manifestations of HIV/AIDS infected persons on antiretroviral drugs (ARDs). Hepatitis C Virus (HCV) infection are known to influence progression and management of HIV infection. Data are limited regarding the impact of ARDs on HIV/HCV co-infected persons in Nigeria. Hence, this study evaluated the biochemical and hematological impact of HCV on prognosis of HIV persons taking ARDs.

Materials and Methods: 2,322 HIV infected persons were screened for HCV. One hundred and nine were co-infected with HCV; and were cross-sectional monitored on ARDs for fifteen months at hospitals in North Central Nigeria for changes in clinical profiles. The determination of Alanine aminotransferase (ALT), Aspartate transaminase (AST), Packed cell volume (PCV) and White blood cells count (WBC) estimations were reviewed every 3 months for each of the person using Reflotron plus machine and hematological analyzer according to the manufacturer's instructions.

Results: The results showed an increase in both HIV mono-infected and co-infected patients, with raised in AST from 18.46±0.73 to 34.32±0.6053U/l, ALT from 19.37±0.6804 to 34.87±0.5637U/l, PCV from 34.20±0.2998 to 34.89±0.4895% and WBC from $3.50x10^9$±0.0816 to $6.67x10^9$±0.1204cells/L and AST from17.35±0.1542 to 34.49±0.0981U/l, ALT from17.67±0.1412 to 34.80±0915U/l, PCV from 36.74±0.2902 to 38.37±0.4399% and WBC from $3.90x10^9$±0.0251 to $6.19x10^9$±0.0178cells/L.

Conclusion: It was found that PCV and WBC count values were positively affected despite HCV replication and AST and ALT enzyme levels for both HIV-mono and co-infected persons were slightly elevated. Therefore, efforts addressing viral hepatitis co-infections at the early stage of ARDs initiation under qualified clinician should be of paramount important.

Keywords: Antiretroviral drugs, Aspartate transaminase, Alanine aminotransferase, White blood cells count, Packed cell volume, Clinico-pathological

1. INTRODUCTION

Nigeria has the largest burden of human immunodeficiency virus (HIV) and viral hepatitis in the world but due to availability of antiretroviral drugs (ARDs), the survival rate has increased. HIV and hepatitis C virus (HCV) are devastating viruses that exhibit similar epidemiological characteristics such as transmission modes and risk populations. These viruses remain commonly found infection with many forms of challenges on individuals, relations, families, communities and the whole society. Their infections are still threatening with a lot of burdens on health care delivering system in Nigeria and other sub-Saharan African countries [1].

It was reported by Kim and his colleagues from their study that individuals infected with HIV are puts at greater risk of co-infection with HCV in relation to the general population [2]. The mechanisms by which the HCV interacts with HIV to impact its disease progression actions are not still properly understood. Although, it has been presumed that co-infection of HIV/HCV may take the advantage of low viral specific CD8+ T cell responses, chronic immune activation and increase in pro-inflammatory cytokines that follow infection by HIV to enter into the host cells [3,4]. Another important challenge is the interaction between HIV and HCV which have a negative impact on liver disease that are caused by these viruses [5,6]. For instance, HCV has been reported to accelerate the evolution and progression of liver disease in HIV- infected individuals [5,7].

There has been recent revival of both biochemical, hematological and political interest in the challenges caused by infection with viral hepatitis C in sub-Saharan African populations where HIV is most endemic [8,9]. The availability and success of antiretroviral drugs (ARDs) has reduced opportunistic infections (OIs) and malignancy in individuals infected with HIV, thereby, improving survival and reducing the emergence of previous unrecognized chronic liver disease caused by viral hepatitis [10,11].

Hepatotoxicity in HIV co-infected patients with viral hepatitis is an important public health concern, with increasing incidence of liver disease [12]. Liver infection is often characterized by biochemical abnormalities function of the liver. Although, some researchers reported that increased serum activity of the two commonly used liver enzymes (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]) may be due to breakdown in the body amino acids, which indicates liver cell injury [13,14]. Opportunistic infections and AIDS related neoplasms are concomitant infection caused by chronic viral hepatitis C that may lead to increase in liver enzyme indices in individuals infected with HIV/AIDS [15,16].

Hematological changes have been found and documented to be one of the common cause of morbidity and mortality in both HIV mono-infected and co-infected patients with HCV. This is marked with changes in cytopoenias such as anaemia, neutropoenia, lymphopoenia and thrombocytopenia [17,18].
Generally, there are several reports on prevalence of co-infection of HIV and HCV that has been documented in Nigeria by some reseachers [19,20], but information is limited on the biochemical (liver enzymes) and hematological indices impact of the HIV/HCV co-infection in the country despite high burden of this viral hepatitis [19]. Therefore, this study was designed to evaluate the biochemical, hematological impact of HCV on prognosis among HIV infected individuals on antiretroviral drugs (ARDs) treatments at the selected hospitals in North Central Nigeria.

2. MATERIALS AND METHODS

2.1 Study areas

This research work was carried out at North Central region of Nigeria. North Central Nigeria (also known as the Middle-Belt region) consisting of six states (Benue, Plateau, Kogi, Kwara, Nassarawa and Niger) and Federal Capital Territory (FCT), Abuja. The population of North central region is over

17.6 million persons and landmass of approximately 273,024Km^2 [21]. The selected hospitals are Federal Medical Centres Keffi, in Nasarawa State and Lokoja, in Kogi State; and General Hospitals Asokoro, in Federal Capital Territory Abuja and Suleja, in Niger State. These hospitals are major treatment and referral centre for HIV infected persons, that received support from the President Emergency Plan for AIDS Relief (PEPFAR) through the Institute of Human Virology in Nigeria.

2.2 Study design

This was a cross-sectional designed study, in which recruitment of the patients was non-randomised and questionnaires were administered on those that consented to participate in the study. The patients infected with HIV were screened for HCV. The HIV mono-infected and HIV/HCV co-infected persons on ART were monitored and followed-up for fifteen (15) months at the selected hospitals.

2.3 Questionnaires

A well-structured self-administered questionnaire was developed to achieved the desire objective of the study and was used to collect information about the socio-demographic characteristics and possible risk factor for transmission of those co-infected. The questionnaire before the study was pretested on 50 HIV infected in the health facilities with the necessary modification and corrections made aftermath of the pre-testing. The socio-demographic variants include age, sex, present place of abode, HIV status, occupation, educational status, history of previous blood transfusion, alcoholism and phone numbers.

2.4 Study population

A total of two thousand three hundred and twenty-two (2,322) HIV infected persons were screened for HCV. One hundred and nine (109) persons were found to be co-infected with HCV. The co-infected persons monitored on ARDs from June, 2013 through February, 2015. In these hospitals, Truvada™, Nevirapine, Combivir™ and Efavirenz were first line (HAART) regimens that were administered.

2.5 Blood collection

A volume of ten millilitres (10mL) of venous blood that were carefully drawn from the veins of each patient and divided into two well labeled Ethylene Diethyel Tetracetic Acid (K_2 EDTA) for hematological parameters (PCV and WBC) assays and plain tube for biochemical parameters (ALT and AST) assays. The assays were carried out according to manufacturer's specifications. The plasma samples were used for screening for HCV antibodies.

2.6 HCV serological screening

The plasma samples of sero-positive HIV patients were screened for antibodies against HCV by rapid ELISA HCV kit ACON (ACON laboratory INC.). The rapid ELISA positive sample were further confirmed using third generation rapid ELISA HCV kit ORTHO HCV ELISA (Ortho-Clinical Diagnostics, Raritan, NJ). These screening were carried out according to the manufacturer's instructions.

2.7 Biochemical assessment

The centrifuged sera obtained from plain tube blood sample was used for biochemical parameters (serum Aspartate transaminase (AST) and serum Alanine aminotransferase (ALT) analysis using

Reflotron plus machine (Rouche Diagnostic GmH, D-68298 Mannhein, Germany) according to manufacturer's instructions.

2.8 Hematological assay

Hematological parameters (PCV and WBC count) were analysed using automated haematology analyzer (Sysmex KX- 21N, Japan). These were done according to manufacturer's instructions.

2.9 Data analysis

Data were collected and analyzed using Statistical Package for the Social Sciences, SPSS (version 19.0). The means±SEM (standard error of mean) of the analytes (biochemical parameters and hematological parameters) and duration (months) were computed. Analysis of variance (ANOVA) was used to test association between means of different variables within the different groups. The level of significance was set at 0.05.

3. RESULTS

From June, 2013 through February, 2015, 2,322 HIV infected and ART-naive patients, age (18 years and above) were recruited and enrolled into this study. Out of these, 1418 (61.1%) were female and 904 (38.9%) were male patients. The ratio of male to female was 1:1.32 and mean age of the study group was 38.0 (18 – 58) years.

3.1 The prevalence of HCV infection among HIV infected patients.

Out of the two thousand three hundred and twenty-two (2,322) HIV infected patients screened for HCV infection, 109 (4.7%) were positive for HCV infection. Of these co-infection 35 (1.5%) were males while 74 (3.2%) were females. The co-infection was highest at age bracket of (28 – 37) years with an occurrence of 52 which represent 2.24% of the total. This was followed by 35 or 1.51% at the age bracket of (38 – 47) years, and 13 or 0.56% at age bracket (18 – 27) years. Age bracket of 58 years and above showed the lowest occurrence of 1 representing 0.04%.

3.2 Socio-demographic characteristics of co-infected patients

The completed questionnaires by the 109 co-infected patients showed that the majority of co-infected persons were females with 74 (3.2%) while the minority were males with 35 (1.5%), $p \leq 0.05$. None of them complained of any form of liver related diseases. Table 1 summarizes the socio-demographic characteristics of co-infected patients. In terms of educational status, tertiary institution was 67 (61.5%) and secondary school were 27 (27.8%), primary school patients 6(5.5%) while uneducated were 9 (8.3%) $p \leq 0.05$. The co-infection rate was higher with married patients 81 (74.3%) compared to single individuals, divorced and widowed populations ($p \leq 0.05$). In relation to age brackets, it was observed that at age brackets of (28 – 37) years had the highest rate of 52 (47.7%) co-infected patients, followed by (38 – 47) years with 35 (32.1%) and (18 – 27) years with 13 (11.9%) ($p \leq 0.05$). Blood transfusion was another status determined, 9 (8.3%) patients were transfused with blood and 100 (91.7%) have not been transfused all their life. The blood transfusion was only observed among the females co-infected patients.

3.3 Biochemical and hematological parameters

The biochemical and hematological parameters among HIV mono-infected showed significant increase during the fifteenth-month followed-up of ARDs use. The mean AST at baseline was 17.35±0.1542 U/l while at the end of fifteenth-month followed-up, the mean of AST was found to be 34.49±0.0981 U/l. The baseline mean ALT was 17.67±0.1412 U/l and 34.80±0.0915 U/l at the end of the followed-up. In this study, it was observed that the (AST and ALT) concentrations among HIV co-infected individuals were higher than concentrations observed in HIV mono-infected patients ($p=0.001$). Similarly, the PCV level at baseline were 36.12±0.0695% and 38.57±0.4399% at the of

fifteen months after initiation of ART while the WBC value was $3.90\times10^9\pm0.0251$ cells/L (p=0.022) at baseline and $7.02\times10^9\pm0.0178$ cells/L (p=0.063) at the end of the study. There were fluctuations observed in PCV during the followed-up period. The increased in both biochemical and hematological parameters obtained in this study was statistically significant ($p\leq0.05$) as in Table 2. Among the co-infected patients, the rate of increase in these parameters were found to be higher when compared with HIV mono-infected persons. The ALT level at baseline was 18.46±0.7367 U/l and 34.32± 0.6053 U/l at the end of fifteen-months followed-up, while the baseline AST was 22.82±0.6375 U/l but rose to 34.87±0.5637 U/l at the end of the fifteen-months monitoring. The increase in biochemical parameters in the two groups were statistically significant ($p\leq0.05$). It was observed prior to the ART initiation, the baseline PCV and WBC counts were 34.20±0.2998% and WBC count was $3.50\times10^9\pm0.0816$cells/L which increased to 34.89±0.4895% and $6.67\times10^9\pm0.1204$ cells/L respectively at the end of the study period. There were fluctuations observed in mean PCV values during the monitoring period, although not statistically significant (p = 0.065), but there was increased in WBC count, AST and ALT and were statistically significant ($p\leq0.05$) shown in Table 3.

Table 1: Socio-demographic and characteristic of naive HIV patients co-infected with HCV at presentation.

Variable	Number (n=109)	Percentage (%)
Sex		
Male	35	32.1
Female	74	67.9
Age brackets		
18-27	13	11.93
28-37	52	47.71
38-47	35	32.11
48-57	8	7.34
>58	1	0.92
Marital status		
Married	81	74.3
Unmarried (single)	22	20.2
Divorced	1	0.9
Widowed	5	4.6
Education		
None	9	8.3
Primary	6	5.5
Secondary	27	27.8
Tertiary	67	61.5
Ever tested for HCV		
Screened	12	11.0
Never screened	97	89.0
Blood transfusion		
Transfused	9	8.3
Never transfused	100	91.7
Alcohol consumption		
Yes	35	32.1
No	74	67.9
Occupational status		
Business	51	46.8
Civil servant	26	23.9
House wife	18	16.5
Others	14	12.8

Table 2: The mean±SEM of biochemical and haematological parameters of HIV mono-infected patients from baseline (0^{th} month) to the end of the follow-up (15^{th} month).

Parameters	Baseline (0^{th} month)	3^{rd} month	6^{th} month	9^{th} month	12^{th} month	15^{th} month	Level of significance
AST (U/l)	17.35±0.1542	19.79±0.1429	22.54±0.1354	25.98±0.1283	28.23±0.1223	34.49±0.0981	p = 0.0001
ALT (U/l)	17.67±0.1412	20.28±0.1327	23.09±0.1324	26.30±0.1254	29.06±0.1109	34.80±0.0915	p = 0.001
PCV (%)	36.12±0.0695	36.74±0.2902	36.27±0.0566	36.52±0.0594	37.03±0.0569	38.57±0.4399	p = 0.003
WBC ($x10^9$)	3.9±0.0251	4.22±0.0158	4.73±0.0181	5.43±0.02080	6.19±0.0202	7.02±0.0178	p = 0.01

AST: aspartate transaminase, ALT: alanine aminotransferase, PCV: packed cell volume, WBC: white blood cells count.

P value at $\leq$0.05 show significant difference.

Table 3: The mean±SEM of biochemical and haematological parameters of HIV co-infected patients from baseline (0^{th} month) to the end of the follow-up (15^{th} month).

Parameters	Baseline (0^{th} month)	3^{rd} month	6^{th} month	9^{th} month	12^{th} month	15^{th} month	Level of significance
AST (U/l)	18.46±0.7367	21.32±0.7409	24.22±0.7223	27.97±0.7473	29.53±0.6785	34.32±0.6053	p = 0.003
ALT (U/l)	19.37±0.6804	22.82±0.6375	26.66±0.6819	29.39±0.7551	28.71±0.6675	34.87±0.5637	p = 0.001
PCV (%)	34.20±0.2998	34.25±0.2731	34.28±0.2681	34.11±0.4350	34.73±0.4623	34.89±0.4895	p = 0.065
WBC ($x10^9$)	3.50±0.0816	3.57±0.0697	3.83±0.0849	4.32±0.1149	5.82±0.1247	6.67±0.1204	p = 0.01

AST: aspartate transaminase, ALT: alanine aminotransferase, PCV: packed cell volume, WBC: white blood cells count.

p value at $\leq$0.05 show significant difference,

4. DISCUSSIONS

As the HIV epidemic in Nigeria continues to pose more challenges despite availability of ARDs in this highly active antiretroviral therapy (HAART) era, new information on HIV/HCV co-infections are needed to guide health care policy makers. HCV infection is one of the serious disease challenges of mankind and worldwide public health concern. The actual clinical impacts of HCV co-infection on the liver enzymes and hematological parameters in HIV infected persons still remains contentious. Nevertheless, some studies have inferred that the presence of HIV infection encourage the course of HCV-related liver infections in co-infected persons [22,23]. In HIV infections, it is proven to compromised T-helper type1 immune reactions which in turn changes the reactions of the immune cells into HCV, which give rise to high HCV replication and higher infection which causes damage to the hepatocytes that leads to hasty progression of HCV-related liver infections (cirrhosis, fibrosis and hepatocellular carcinoma) [22].

In this cross-sectional study, we examined the biochemical and hematological impact of HCV on HIV infected individuals on antiretroviral therapy (ART). ART has been accepted as gold standard for the treatment of HIV/AIDS and has helped in changing the thinking of infected individuals from an incurable-disease to manageable chronic disease [24,25]. Although, despite the standard treatment options, HIV infection has been documented to cause several degrees of immune-pathogenesis in human and this carries enormous biochemical and hematologic changes [26]. The hematological consequences of HIV infection are mostly cause by peripheral blood cytogenesis (eg anemia) which have become more apparent during this era of HAART.

In this present study, there was significant increase in serum liver marker enzymes (AST and ALT) in both HIV mono-infected and co-infected patients from baseline through fifteenth month followed-up, this may be due to associated hepatotoxicity and HCV infection. This observation is in agreement with that of other workers [27,28] who observed in their studies an association between positive anti-HCV and elevated serum AST and ALT. It was also observed that AST and ALT activity were significantly higher among co-infected patients at the baseline to the end of followed-up compared to HIV mono-infected individuals ($p \leq 0.05$). This finding showed some problems to be encountered in treating patients who were co-infected, especially regarding which HAART regime to be administer, how to prevent further hepatic damage, and when to initiate HAART, in low-resource settings like Nigeria with scarce of ART alternatives. For instance, protease inhibitors (PIs) have no any impact on HCV and may cause progressive increase of AST and ALT concentrations [5,7]. It is proven that HIV worsens HCV infection, thereby causing severe cirrhosis, fibrosis and finally death arises from liver complication and disease [5,7]. Therefore, it is of paramount important to make HCV antibody screening for every HIV infected persons before initiation ARDs and monitoring of liver enzymes abnormalities and hematological parameters on regularly basis during ARDs usage in both HIV mono-infected and co-infected persons.

In this study, PCV showed a consistent significant increased in value during the followed-up period, although the reduction at the 3rd month was significant $p \geq 0.05$ for co-infected patients (Table 3). There is reduction from 3rd to 6th month (Table 2) and from 6th to 9th month observed in PCV value for HIV mono-infected and co-infected patients respectively. This fluctuation or reduction observed in the mean PCV value may indicate suppressive activity of the ARDs on the virus or opportunistic infections or mild anemia with the continual usage of these drugs. These abnormalities may also occur in patients as a result of the following actions, HIV infection, sequel of HIV-related opportunistic infections (HIV), malignancies and challenges of treatments used for HIV infection and associated conditions [29].

White blood cell (WBC) count was another important parameter monitored in this study, because it was reported that infections like HIV or its co-infection with HCV, that elevation in WBC counts may indicate infection, lack of response to treatment, ARDs or an abnormality. In this present study the white blood cells count (WBC) were reviewed after every three months for fifteen months and it was

found that WBC increase was statistically significant ($p \leq 0.05$) in both HIV mono-infected and co-infected patients. Such could be due to the functional betterments of these two important components of innate antimicrobial immunity, i.e. neutrophils and monocytes, that may also contribute to the improved cell-mediated immune responses against other infections in HIV/AIDS patients treated with various HAART regimens as observed in this study. This was also similar to observations made by Derbe and his co-workers in 2011 [30] in their study, who reported that in responses against other infections in HIV/AIDS treated individuals on various HAART regimens, there is significance elevation in WBC count that could have help in reducing risk of other opportunistic infections.

Generally, this present study supports the earlier findings of Chukwurah and his co-workers in 2007 [31], who reported in their study that the use of ARDs has ability to increased PCV, Hb, WBC and CD4+ count and hence, boosting the body immune system despite the reported side effect of the ARDs. Therefore, the ARDs usage and efficacy would become more apparent if proper management would be initiated at the early stage of treatment and under qualified clinician.

5. CONCLUSIONS

We concluded from our finding that HCV infection has clinical impacts on both biochemical and hematological parameters of patients on ARDs. Therefore, it is recommended to make HCV antibody screening paramount important for every HIV infected persons before initiation ARDs and monitoring of liver enzymes abnormalities and hematological parameters on regularly basis during ARDs usage in both HIV mono-infected and co-infected persons. This may have helped in achieving the desired goal of the treatment.

CONSENT

Informed and written consent was obtained from all individual patients included in the study.

ETHICAL CONSIDERATIONS

Ethical approval for the study was secured and obtained from the Health Research and Ethics Committees of the selected hospitals where the study was carried out in North Central region of Nigeria, in accordance with the code of ethics for biomedical research involving human subjects. The patients were recruited after they were sufficiently counseled on the objectives, risk and importance of the study. All relevant confidentiality was kept throughout and after the study period.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author YY did the conceptualization, proposal writing and literature searches, discussion/choice of method, supervised bench work and writing up manuscript for publication. Author SBM did the literature searches, proposal writing proof reading, discussion/choice of method, data analysis and supervised bench work. Author NJF supervised and collect data from various facilities, Proof read the manuscript. Author OKT carried out literature searches, socio-demographic data collection, bench work and data analysis. Author AA did the sample collections, transportation of samples and bench work. Author UM did the socio-demographic data collection and bench work. Author DOC carried out data entering and statistical analysis. Author ARA carried out socio-demographic data collection, bench work and data entering.

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