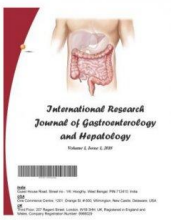

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Association between Hepatitis C Virus Infection and the Immunologic, Hematologic Parameters in HIV-Positive Adults in a Tertiary Health Facility in Southeastern Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author BOA designed the study, wrote the protocol, wrote the first draft of the manuscript and performed the statistical analysis. Author EOO managed the literature searches and did the sample collection. Both authors equally participated in the sample analyses. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Three-quarters of liver-related diseases and deaths are caused by Hepatitis C virus (HCV) infection among human immunodeficiency virus (HIV) infected individuals. Data on HCV and HIV co-infection in Nigeria in general and Umuahia metropolis, in particular, are scarce. Thus, to close this gap, we examined the relationship between HCV- infection and the immunologic, hematologic profile of HIV- infected patients, and determined the prevalence of HCV infection among patients.
Methods: This cross-sectional study examined 143 blood samples of HIV-infected patients 18 years and older collected between August 2016 and November 2016 in the ART clinic of the Federal Medical Centre, Umuahia, Nigeria. We analyzed samples for HCV antibodies using the rapid ELISA

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technique; CD4 count using the Cyflow machine, white blood cell count (WBC) and packed cell volume (PCV) using the Sysmex machine. Prevalence and associations were determined using frequency distribution and $\chi 2$ test and α level set at .05.

Results: One hundred and eighteen (83%) subjects were on ART and 25 (18%) non-ART. Fifty two (36%) subjects were males and 91 (64%) were females. The age range was 18 - 71 years and a mean of 40.64 years. HCV prevalence was 14%. Prevalence by gender was 11% male and 15% female. Prevalence by ART status was 60.0% ART and 3.4% Non-ART. There was statistically significant associations between HCV and ART status ($\chi 2 = 32.3$, $P = .001$); between HCV and CD4 cell count ($\chi 2 = 8.87$, $P = .031$) but no associations between HCV and WBC ($\chi 2 = 1.615$, $P > .05$) and PCV ($\chi 2 = 1.002$, $P > .05$). Prevalence was more in the CD4 ≤ 200 cells/ml groups (40%) and the 31-50 age groups (40.0%).

Conclusion: HCV/HIV co-infection was associated with low CD4 cell count and being female. It is therefore essential to maintain the CD4 cell of HIV-positive individuals at a number> 300 cells/ml to prevent possible co-infection with HCV, and this will help avoid additional disease and financial burden on people living with HIV/AIDS in Nigeria.

Keywords: Hepatitis C virus; Immunologic; Hematologic parameters; HIV-infection.

1. INTRODUCTION

Hepatitis C virus (HCV) is an RNA flavivirus that infects 130-150 million people globally [1], and a significant cause of chronic liver disease worldwide [2,3]. World Health Organization (WHO) estimated that 3-4 million new HCV infections occur yearly and that the prevalence of HCV in Sub Saharan Africa is 5.3% [4] and 3.6%-5% in Nigeria [5,6]. HCV is a significant public health burden, especially in sub-Saharan Africa. It is estimated that more than one-quarter of HIV-infected individuals are co-infected with the HCV globally and that HCV infection causes three- quarters of liver-related diseases and deaths among HIV infected patients. The prevalence of HBV and HCV among individuals at risk for or living with HIV is because the mode of transmission of these viruses is similar in some ways to the ways HIV is transmitted [7].

HCV is blood borne and transmitted mainly by transfusion of unscreened infected blood and blood products, needle stick injury, tissue, and organs transplant [8]. Other routes of transmission include sexual intercourse, vertical and intravenous drug use [9].

Studies showed that acute infections are clinically asymptomatic, and only about half of patients with viremia show increased Alanine Aminotransferase activity. As a result of the asymptomatic and slow onset of HCV infection, most patients are unaware of their infected status; hence HCV infections are most times detected late when the chronic stage had set in, and treatment options are already limited [10]. According to WHO, approximately 700 000 people die annually from HCV infections with a majority of the people being unaware of their status, thereby increasing the chances of developing liver disease and also transmitting the virus to others.

HCV hepatitis disease progression among HIV-infected individuals is faster and causes more liver-related problems than among non-HIV infected individuals [11]. Leen also stated in his study that HCV infection might impair the response of CD4+ cells to anti-retroviral therapy (ART) treatment [11]. According to AIDS.gov, individuals with HCV/HIV co-infection are more than three times the risk for liver disease and liver-related death, and that HCV/HIV co-infection can complicate the management of HIV infection. A cross-sectional study showed that HIV-infected women were nearly two times as likely as HIV-uninfected women to be infected with HCV (adjusted odds ratio, 1.9; 95% confidence interval, 1.2–2.9 [12]. Though the biologic explanation for faster viral hepatitis disease progression in HCV/HIV co-infection is not fully understood, more rapid disease progression may be related to the responses of impaired T-cell to HCV and the effect of HIV on hepatic cells [13].

The immunopathogenesis of viral diseases involves the production of a cytokine by the host in response to viral infections. However, the co-existence of two chronic viral infections, as in the co-infection of HCV and HIV alters the profile of cytokine production. The co-infection of HCV and HIV decreases the IFN-γ and TNF-α expression significantly and also increases the production of IL-10 production in vitro [14]. Antiviral cytokines

such as IFN-γ and TNF-α are said to mediate the clearance of the virus. However, due to the decrease in the production of IFN-γ and TNF-α and elevated IL-10 levels, the infections persist with disease progression [15]. Co-infection increases the financial burden, reduces productivity, and increases the disease burden of hepatotoxicity in HIV- infected patients [16].

Data on HCV and HIV co-infection in Nigeria in general and Umuahia metropolis, in particular, are scarce. A few studies were conducted in southwest Nigeria [17] and Mid-west Nigeria [18] that showed that there is a pathological struggle for the hepatocytes in the livers of patients with viral co-infection and more massive burden on the immune system of the individual with dual viral infection. We, therefore, hypothesized that HCV/HIV co-infection places more onerous burden on the immune and the hematologic profiles of the patients. To test this hypothesis, we determined the prevalence of HCV and explored the relationship between HCV and the immunologic, hematologic statuses of HIV-infected individuals.

2. MATERIALS AND METHODS

2.1 Sample Size

The sample size was determined to be 143 using the G* Power software [19]. The sample size was calculated using the following parameters of G* Power software: $\chi 2$ test, the effect size of 0.3, a power of 0.80, and 5 degrees of freedom (Df). We collected samples systematically from one out of every three subjects until we reached the sample size of 143 participants.

2.2 Procedure

We collected four millilitres of venous blood samples from each of the one hundred and forty-three HIV-positive individuals. Two millilitres each of the samples were collected into sterile appropriately labelled plain blood collection tubes to allow blood clotting and serum separation, whereas, two millilitres each of the samples were collected into sterile appropriately labelled ethylenediaminetetraacetic acid (EDTA) bottles to prevent blood clotting. The samples were then taken to the ART laboratory for analyses. We used the sera for the HCV antibody tests and the anti-coagulated blood samples for both the CD4+ cell count and white blood cell counts (WBC) analyses.

Every form of identifier that could link the samples to the subjects was removed before sera were analyzed for HCV antibodies using the rapid test enzyme immunoassay (EIA) rapid technique (FSC –ISO 13485 HCV test kit). CD4+ cell counts as the immunologic parameter were analyzed using the Partec Cyflow machine. White blood cell counts (WBC) and packed cell volume (PCV) representing the hematologic parameters were analyzed using the Sysmex haematology analyzer. The deductive reasoning theory was applied in this study to deduce the associations among the variables.

2.3 Statistical Analysis

Data were entered manually into Microsoft Excel and then exported into SPSS version 23 for analyses after inspection and coding. Descriptive statistics using the frequency distribution of demographic and laboratory test values were used to determine the Sero-prevalence of HCV, whereas the associations between the nonparametric variables were assessed using the Chi-square test. Statistical significance was set at $p < 0.05$. The deductive reasoning theory was applied in this study to examine the associations among the variables.

2.4 Ethical Approval

This study was approved by the health research ethics committee (HREC) of Federal Medical Centre Umuahia. Following approval by HREC, informed consent was then obtained from the subjects before sample collection.

3. RESULTS AND DISCUSSION

The distribution of data showed that HIV-positive individuals on antiretroviral therapy (ART) were 118 (82.5%) while those who were yet to be enrolled for ART were 25 (17.5%) (Table1). Of the 143 study subjects, 52 (36.4%) were male, and 91 (63.6%) were female (Table 1). The age ranged between 18 and 71 years with mean age of 40.64±10.7 years, mean CD4+ 318.9±212.1, mean WBC 5.4±1.5, and mean PCV 40.8±36.9 (Table 1). Twenty subjects tested positive for HCV, giving overall HCV Seroprevalence of 14% (Table 1).

HCV Seroprevalence by gender, age, and ART status is shown in Table 2. HCV seroprevalence was more among females (15.4%) than in males (11.5%). Chi-square $\chi 2$ value of 0.41, $P = .52$ showed that there was no significant association

between gender and HCV infection. (Table2). Prevalence was also more in the 31-50 years age groups (40.0%), followed by group 18-30 years group (35%) and no positive reaction in the >60 years group (0.0%). χ2 6.34, P = .09 also indicated no significant association between age and HCV infection (Table 2). However, there was a significant association between ART status and HCV (χ2 32.3, P = .001). HCV seroprevalence was more with patients on ART (64.0%) than non-ART (3.4%) patients.

Table 3 is results of the relationship among HCV, CD4+, WBC, and PCV. Seroprevalence was more in the CD4+ ≤ 200 cells/ml groups (40%), followed by the 201-300 group (35%) and the 301-500 (25%), and no observed reaction to HCV in the >500 group (0.0%). There was a statistically significant association between HCV and CD4 cell count (χ2 = 8.87, P = .03) but no association between HCV and WBC (χ2 = 1.615, P = .45) and PCV (χ2 = 1.00, P =. 61) (Table 3).

3.1 Discussion

This cross-sectional, descriptive, and observational study was aimed at determining the association between hepatitis C virus infection and the immunologic, hematologic parameters in HIV-infected adults attending the anti-retroviral therapy clinic in Federal Medical Centre Umuahia (FMCU). The prevalence of HCV infection in the study population was also determined. HCV and HIV co-infection have been documented in similar earlier studies [20, 21,22]. In this study, 14% of the HIV-infected individuals were co-infected with HCV. This figure is comparable to the 14.7% reported in Lagos, southwest Nigeria [20] and 13.2% recorded in the United States [21]. However, lower figures were reported in Midwest and north-central Nigeria (7.0% and 8.2% respectively [18,22].

This study also showed a higher seroprevalence of HCV infection (40%) observed in the patients with CD4+ cell count ≤ 200 cells/ml compared to that observed in patients with CD4+ cell count range 201-300, 301-500 (35%, 25%) respectively and no observed seroprevalence in the CD4+ >500 range (0.00%). Furthermore, the statistically significant association between CD4+ cell count and HCV infection (χ2= 8.87, P = .03) obtained in this study is consistent with the results of Ojide et al. with respect to the observed higher HCV seroprevalence (8.0%) in patients with CD4+ cell count ≤200cells/ml compared to the observed lower rate of 6.0% in patients with CD4+ cell count > than 200cells/ml. However, their study recorded no statistical significance [18]. Furthermore, the more Seroprevalence of HCV among ART patients (64.0%) recorded in this study compared to non-ART patients (3.4%) could be due to the already weakened immune system of the HIV patients on ART, since HIV-infected patients are only initiated into ART when CD4+ cell count is <350. According to the reports of Greub et al., synergies exist between HCV infection and HIV disease progression [23]. These synergies may also account for the more HCV prevalence recorded among ART patients.

Table 1. Characteristics of study population

Variable	Frequency (%)
Gender	
Male	52 (36.4)
Female	91 (63.6)
Age group (years)	
18-30	30 (21.0)
31-50	87 (60.8)
51-60	21 (14.7)
>60	05 (3.5)
Mean age	40.6±10.7
ART Status	
ART	118 (82.5)
Non ART	25 (17.5)
CD4+cell count (cells/ml)	
≤200	49 (34.3)
201-300	31 (21.7)
301-500	40 (28.0)
>500	23 (16.1)
Mean CD4+ count	318.9±212.1
HCV Status	
Reactive	20 (14.0)
Non-Reactive	123 (86.0)
WBC count (cells/µl)	
2-4	28 (19.6)
4.1-6.0	67 (46.9)
6.1-10	48 (33.6)
Mean WBC	5.4±1.5
PCV (%)	
14-30	9.0 (6.3)
31-50	131 (91.6)
>50	3.0 (2.1)
Mean PCV	40.8±36.9

Table 2. Seroprevalence of HCV by gender, age, and art status

Variable	HCV Status		χ^2	*P*-value	Sig.
	Reactive (%)	Non-reactive (%)			
Gender			0.41	.52	NS
Male	6.0 (11.5)	46.0 (88.5)			
Female	14.0 (15.4)	77.0 (84.6)			
Age group (years)			6.34	.09	NS
18-30	7.0 (35.0)	23.0 (18.7)			
31-50	8.0 (40.0)	79.0 (64.2)			
51-60	5.0 (25.0)	16.0 (13.0)			
>60	0.0 (0.0)	5.0 (4.1)			
ART Status			32.3	.001	S
ART	16.0 (64.0)	9.0 (36)			
Non -ART	4.0 (3.4)	114.0 (96.6)			

Note: HCV= hepatitis C virus; ART= Anti-retroviral therapy; non-ART= non Anti-retroviral therapy; χ^2 = Chi square; Sig= significance; NS= not significant

Table 3. Relationship among HCV, CD4+ cell count, WBC, and PCV

Variable	HCV Status		χ^2	*P* -value	Sig.
	Reactive (%)	Non-Reactive (%)			
CD4+cell count (cells/ml)			8.87	0.03	S
≤200	8.0 (40.0)	41.0 (33.3)			
201-300	7.0 (35.0)	8.0 (40)			
301-500	5.0 (25.0)	35.0 (28.5)			
>500	0.0 (0.0)	23.0 (100)			
WBC count (cells/µl)			1.62	0.45	NS
2-4	3.0 (15.0)	25.0 (20.3)			
4.1-6.0	12.0 (60)	55.0 (57.6)			
6.1-10	5.0 (25.0)	43.0 (35.0)			
PCV (%)			1.00	0.61	NS
14-30	2.0 (1.4)	7.0 (4.9)			
31-50	18.0 (12.6)	113.0 (79.0)			
>50	0.0 (0.0)	3.0 (2.1)			

Note: HCV= hepatitis C virus; χ^2 = Chi square; Sig= significance; NS= not significant
S= significance.

It is said that CD4+ cell count <200cells/ml have no significant response to anti- HCV therapy. As a result, it is advisable that HIV and HCV co-infected patients be treated with highly active antiretroviral therapy (HAART) first to boost the immune system to 500 cells/ml and above before initiating anti-HCV treatment.

In this study, there were more HCV seropositive cases (15.4%) in HIV-positive females than their male counterparts (11.5%). This report agrees with the results recorded in similar studies in Lagos Nigeria and Tanzania respectively [17, 24]. Similarly, a cross-sectional HCV prevalent study found that HIV-infected women were almost twice as likely as HIV-uninfected women to acquire HCV (adjusted odds ratio, 1.9; 95% confidence interval, 1.2–2.9 [25]. This report suggests that HIV-infected women may be more susceptible than non-HIV-infected women.

The high prevalence of HCV and HIV co-infection recorded in this study could be because both viruses share similar routes of transmission. However, to confirm this report, more studies are needed. Furthermore, as a result of the results obtained in this study, there is the need for routine and regular screening for HCV among HIV-infected individuals for early detection of HCV co-infection. It is worthy to note that a missed diagnosis of HCV infection may lead to ill-informed treatment decisions and poorer treatment outcomes for both HCV and HIV infections because dual infection automatically alters the management of both diseases. Thus, early detection and initiation of anti-HCV therapy can prevent liver necrosis and end-stage liver

disease and subsequent death in HIV-positive individuals.

3.2 Study Limitation

This study is limited in the aspect that we conducted only commercial HCV antibody screening. HCV RNA confirmatory testing by polymerase chain reaction was not done. Also, due to the use of the ELISA test, there may be false negative cases in immunosuppressed individuals with hepatitis C viremia [26].

4. CONCLUSIONS AND RECOMMENDATIONS

Study showed that HCV/HIV co-infection was associated with low CD4+ cell count. There is also the tendency that HIV patients on ART are more prone to co-infection with HCV than the non-ART patients. The above observations showed that there is an association between the immune statuses of HIV-positive individuals and HCV infection. Also, being female was a factor to reckon with in the seroprevalence of HCV infection in this study. However, there was no association observed between HCV infection and the hematologic parameters studied in HIV-infected individuals. Thus, with this knowledge, it is crucial that the CD4 cell count of people living with HIV/AIDS (PLWHA) be maintained at a count > 300 cells/ml to prevent possible co-infection with HCV. Also, regular screening for HCV among HIV/AIDS patients will give room for proper management if detected early. Early detection will help prevent additional disease and financial burden on this population groups (PLWHA) in Nigeria. It is recommended here that screening for HCV should be conducted on all HIV-infected individuals with an anti-HCV antibody test on entry into HIV care. Testing for HCV should not be deferred because of an absence of symptoms or elevated hepatic transaminase levels, Since HCV infection is often not readily detectable clinically (clinically silent) until late stages when transaminase levels are significantly elevated enough to trigger screening.

ETHICAL APPROVAL AND CONSENT

This study was approved by the health research ethics committee (HREC) of Federal Medical Centre Umuahia. Following approval by HREC, informed consent was then obtained from the subjects before sample collection.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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