



Zero Prevalence of HIV and HCV Coinfection in the Highly HIV-infected Population of Rivers State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author IOO designed the study, performed the statistical analysis and wrote the protocol. Author TIC managed the analyses of the study. Authors TIC, IOO and NFP managed the literature searches and wrote the first draft of the manuscript. Authors IOO and NFP supervised the whole study which, Author TIC used as part of her Ph.D. Thesis in the Department of Microbiology, University of Port Harcourt, Nigeria. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRIMPS/2021/v10i330164

Editor(s):

(1) Dr. Davina Hijam, Regional Institute of Medical Sciences, India.

Reviewers:

(1) Nicholas Jairo Kavana, St. Francis University College of Health & Allied Sciences, Tanzania.

(2) Eugenia Nonye Ojiako, Chukwuemeka Odumegwu Ojukwu University, Nigeria.

Complete Peer review History: <https://www.sdiarticle4.com/review-history/69230>

Original Research Article

Received 01 April 2021
Accepted 03 June 2021
Published 07 September 2021

ABSTRACT

Background: Hepatitis C affects 5–15% of the 38 million people living with HIV globally. Africa which has the second highest prevalence of HIV/HCV co-infection following Asia. This alarming statistics has made it crucial that studies be done to also ascertain the HIV/HCV co-infection prevalence in the country and host factors which may influence the co-infection.

Objective: Therefore, this study was conducted to investigate the seroprevalence of HCV infection amongst the HIV population of Rivers state, Nigeria.

Study Design: Cross-sectional study.

Place and Duration of Study: University of Port Harcourt Teaching Hospital (UPTH), in Rivers State, Nigeria, from February 2017 to September 2019.

Methods: In this study, 226 HIV-infected individuals were recruited comprising 105 males and 121

females. These subjects were screened for the presence of HIV and HCV using ELISA and was performed according to the kit manufacturer's stipulations. The demographic characteristics of the participants were obtained using a questionnaire designed for the study.

Results: The presence of antibodies to HIV-1 reconfirmed the HIV status in all the study subject. A seroprevalence rate of 0.0% was observed for HIV/HCV infection. The ratio of females to males was found to be 1.2:1. The ages of the study population ranged from 16 to 70 years with a median age of 42.5 years. A large number (30.5%, n = 69) of the participants were within the age group 40 – 49 years, followed by those within 30 – 39 years (26.1%, n= 59), 50 – 59 years (17.3%, n = 39) and >59 years (13.7%, n = 31). Participants that were within younger age groups had less population; 20 – 29 years (9.7%, n = 22), with those that are <20 years having the least population (2.7%, n = 6). Many (49.1%, n = 111) of the study participants were found to be married. About 46.5% (n = 105) of them were single, while a few (4%, n = 10) were widowed.

Conclusion: No coinfection of HIV/HCV was found, in spite of Nigeria being endemic for HCV. However, despite this zero rate of HIV/HCV coinfection, routine screening for HCV markers should be carried out to reduce morbidity and mortality in HIV-infected individuals.

Keywords: Antibodies; HIV; HBV infection; Co-infections; Nigeria.

1. INTRODUCTION

Hepatitis C affects 5–15% of the 38 million people living with HIV globally [1]. Africa which has the highest percentage of people living with HIV also has the second highest prevalence of HIV/HCV co-infection following Asia [2]. Nigeria which has the second largest HIV epidemic in the world accounts for two-thirds of new HIV infections that occurred in 2019 in West and Central Africa. The country also accounts for about half of all new HIV infections that occur in sub-Saharan Africa every year together with South Africa and Uganda [3] [4] and is amongst the countries that record the highest rates of new infection in sub-Saharan Africa.

This alarming statistics has made it crucial that studies be done to also ascertain the HIV/HCV co-infection prevalence in the country and host factors which may influence the co-infection. Both viruses which share the same modes of transmission commonly co-occur and a large percentage of individuals are afflicted with both infections. The co-infection of a patient with both viruses means an accelerated progression of HCV ultimately resulting in liver-related morbidities and mortality [5]. Awareness of the possible co-existence of both viruses in a patient is important when choosing a drug regimen as HIV/HBV co-infected patients have been shown to be less responsive to HBV treatments and have high risks of drug interactions [6-7].

Knowledge of the prevalence of the co-infection will guide in the education of the population on the need of a healthy lifestyle to avoid contracting both viruses. This knowledge is also essential for the formulation of prevention

strategies, for guidance in clinical care, monitoring and treatment of infected patients and future research. Therefore, this study was conducted to investigate the seroprevalence of HCV infection amongst the HIV population of Rivers state, Nigeria. The demographic characteristics of the patients such as age, sex and marital status were also noted to screen for the existence of any pattern of distribution of the co-infection amongst the patients.

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted among HIV positive individuals attending the Anti-Retroviral Clinic (ARV) of the University of Port Harcourt Teaching Hospital (UPTH). UPTH is a major tertiary-care teaching and research facility owned and managed by the Federal Government of Nigeria. It is located in the Alakahia community along East-West road, Obio-Akpo Local Government of Rivers State, Nigeria. Rivers State is in the south-south geopolitical zone of Nigeria and has 23 Local Government Areas. It is bounded by the Atlantic Ocean to the south, by Imo, Abia and Anambra States to the North, by Akwa-Ibom State to the East and by Bayelsa and Delta States to the West. The State has a total population of about 5.19 million, according to the 2006 census data with a growth rate of 3.0% ("Nigeria: Administrative Division", n.d.), making it the 6th most populous states in Nigeria. This is not surprising as it is economically significant as the centre of Nigeria's oil industry. Rivers State is home to many ethnic groups: Ikwerre, Ijaw, Kalabari, Etche, Ogba, Ogoni

and others. Rivers State with a HIV/AIDS prevalence of 3.8% ("Rivers State", n.d.), the third most prevalent in the country, is not exempt from the HIV/AIDS pandemic prevalent areas in Sub-Saharan Africa.

2.2 Study Design

A cross-sectional survey design was adopted for the present study which sought to evaluate the dual seropositivity of HIV and HCV coinfection among HIV-infected individuals in University of Port Harcourt Teaching Hospital in Port Harcourt, Nigeria. Random sampling was used to select consenting participants for the study from whom blood samples were collected. Descriptive statistics such as frequencies, percentages, tables and charts were used to represent data obtained.

2.3 Study Population

The study population constituted HIV seropositive individuals under clinical monitoring in the antiretroviral clinic in the University of Port Harcourt Teaching Hospital (UPTH) from February 2017 to September 2019. A total of two hundred and twenty-six (226) participants were selected and enrolled for the study (Table 1).

2.4 Inclusion and Exclusion Criteria

All consenting individuals aged 18 years and above with positive HIV diagnosis attending HIV clinic in UPTH were recruited for this study. Patients who did not have their HIV status confirmed, who were seronegative and who did not give their consent were excluded from partaking in this study.

2.5 Sampling Technique

From the study population, a total sample size of 226 HIV positive individuals were randomly selected and enrolled into the study. The demographic details (age, gender and marital status) relevant to the study were obtained from the hospital records. The information obtained were stratified as follows; the gender was classified as males (M) and females (F); the ages of the participants were grouped into six: <20 years, 20-29 years, 30-39 years, 40 – 49 years, 50 – 59 years and >59years while the marital status of the patients was classified as married, single or widowed.

2.6 Sample Collection, Preparation and Storage

Upon obtaining written consents from the study participants, specimens of venous blood (about 5ml) were aseptically drawn from them into sterile EDTA (ethylene diamine tetra acetic acid) tubes. The blood was allowed to separate, and the plasma aspirated into sterile Eppendorf tubes. Samples were clearly identified with codes in order to avoid misinterpretation of results. Haemolysed and visibly hyperlipemic samples were discarded as they could generate false results. Samples were stored in two aliquots at -20°C and -80°C pending serological analysis.

2.7 Serological Analysis

2.7.1 HIV Ab and Ag assay

The HIV status of the participants in the study was reconfirmed by screening their plasma samples for the presence of HIV type 1, 2, and P24 HIV-1 antibodies, using commercially available Enzyme Linked Immunosorbent Assay (ELISA) manufactured by DIA.PRO Diagnostic Bioprobes Srl Via G. Carducci n° 27 20099 Sesto San Giovanni (Milano) – Italy. The test results were calculated by means of a cut-off value determined by the following formula on the mean OD450nm value of the negative control (NC): $\text{Cut-Off (Co)} = \text{NC} + 0.125$. The value found for the test was used for the interpretation of results. Test results were interpreted as the ratio of the sample OD450nm (S) and the Cut-Off value (Co), mathematically S/Co . Values less than 1 indicated a negative reaction while values greater than 1 indicated a positive reaction. A negative result indicates that the patients have not been infected with HIV. A positive result was indicative of HIV infection and therefore the patient should be treated accordingly.

2.7.2 Anti-HCV assay

Serum antibodies against HCV antibodies were analyzed *in vitro* using a commercial kit (DIA.PRO Diagnostic Bioprobes, Milano, Italy) based enzyme-linked immunosorbent assay (ELISA). The serologic test and interpretation of results were done according to instructions of the kit manufacturer. Optical signals generated in the microwells were read at 450 nm with an ELISA microplate reader (Model ELx808i, BioTek Instruments, USA). The ELISA kit manufacturer provided the formula for calculating the cut-off OD450nm (OD of negative control plus 0.250)

which we used as a threshold for determining the reactive and non-reactive serum samples. The cut off value was calculated using the mean OD450nm value of the Negative Control (NC): NC + 0.350 = Cut-Off. Samples with an OD450nm lower than the Cut-off value were considered not reactive for antibodies specific to the HCV antigens present in the vaccine. Samples with an OD450nm higher than the Cut-Off value were considered positive for antibodies specific to the HCV antigens present.

2.8 Statistical Analysis of Data

Descriptive analysis was performed to summarize data obtained. Differences among groups were compared using Chi-square tests for categorical variables. P values <0.05 were considered statistically significant. Data analysis was done using IBM SPSS Statistics, version 22 (IBM Corp).

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Analysis of the study population

Characteristics of the study group are highlighted in Table 1. Many (53.5%, n = 121) of the participants of the study were females while 46.5% (n = 105) of the study population were males. The ratio of females to males was found to be 1.2:1. The ages of the study population ranged from 16 to 70 years with a median age of 42.5 years. A large number (30.5%, n = 69) of the participants were within the age group 40 – 49 years, followed by those within 30 – 39 years (26.1%, n= 59), 50 – 59 years (17.3%, n = 39)

and >59 years (13.7%, n = 31) as seen in Table 1. Participants that were within younger age groups had less population; 20 – 29 years (9.7%, n = 22), with those that are <20 years having the least population (2.7%, n = 6). Many (49.1%, n = 111) of the study participants were found to be married. About 46.5% (n = 105) of them were single, while a few (4%, n = 10) were widowed.

Table 1. Demographic characteristics of HIV-infected individuals

| Variables | Number Tested | Percentage (%) |
|---------------------------|---------------|----------------|
| Age groups (Years) | | |
| <20 | 6 | 2.7 |
| 20 – 29 | 22 | 9.7 |
| 30 – 39 | 59 | 26.1 |
| 40 – 49 | 69 | 30.5 |
| 50 - 59 | 39 | 17.3 |
| >59 | 31 | 13.7 |
| Sex | | |
| Males | 105 | 46.5 |
| Females | 121 | 53.5 |
| Marital Status | | |
| Married | 111 | 49.1 |
| Single | 105 | 46.5 |
| Widowed | 10 | 4.4 |
| Total | 226 | 100 |

3.2 Overall Seroprevalence

A total HIV seropositivity of 100% was obtained for all samples, reconfirming the HIV status of the study participants. Further analysis for HCV antibodies using the ELISA kit manufactured by DIA.PRO Diagnostic Bioprobes, Milano – Italy revealed prevalence rates of 0.0% for HCV (Fig. 1).

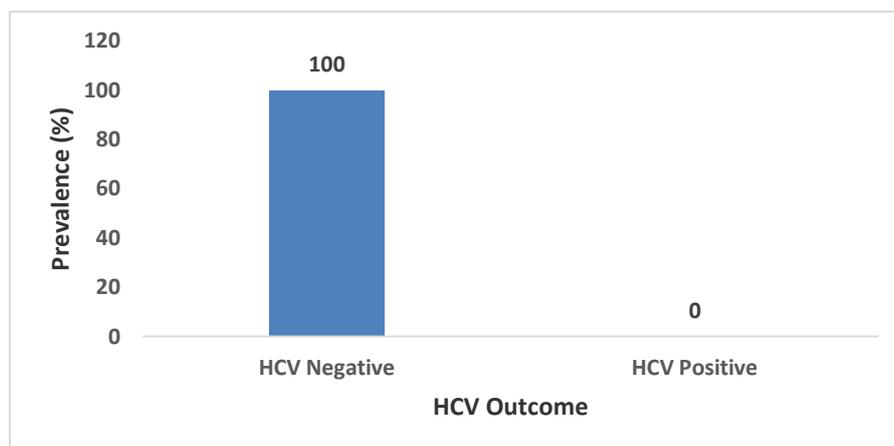


Fig. 1. Seroprevalence of Anti-HCV antibodies among HIV infected individuals

3.3 Discussion

3.3.1 Demographic characteristics of patients

The median age of the study population was found to be 42.5 years suggesting that many of the subjects were middle aged. It was observed that the proportion of the study population increased with increasing age groups, up to 40 – 49 years, after which it declined with an increase in the age group. The largest proportion (30.5%) of the study participants were within the age group, 40 – 49 years while the least was found within the youngest age group, <20 years (2.7%). Many (53.5%) of the participants of the study were females while 46.5% of the study population were males. This observation agrees with Ekere *et al.* [8] and Okonko *et al.* [9-10], who also found the majority of HIV-infected subjects to be females. The higher percentage of females observed in this study could be attributed to females being at a higher risk for HIV infection in developing countries especially in sub-Saharan Africa [10]. Also, based on the findings by the Nigeria HIV/AIDS indicator and impact survey (NAIIS), the National Agency for the control of AIDS in 2019 reported that more females (1.9%) than males (0.9%) were living with HIV/AIDS in Nigeria [11- 12]. Many (49.1%) of the study participants were found to be married while about 46.5% of them were single and a few (4%) were widowed. This is in contrast to studies by Shisana *et al.* [13], Kposowa [14] and Tlou [15] who revealed that widowed, divorced and single individuals are at higher risk of contracting HIV infection than their married counterparts.

3.3.2 HIV coinfections with HCV

A total HIV seropositivity of 100% was obtained for all samples, reconfirming the HIV status of the study participants. HIV has been reported to be associated with a higher prevalence of both Hepatitis B and C in Sub-Saharan Africa and these infections share a common mode of transmission with HIV [16]. In Nigeria, hepatitis co-infection with HIV is linked with an increase in morbidity and mortality [17]. Consequently, coinfections with C was assessed.

The 0.0% coinfection rate with HCV contradicts the 15.0% and 23.5% reported by [18-19] respectively, in Abeokuta, Ogun State and Ughelli, Delta State. Elsewhere, Tsai *et al.* [20] reported a 4.7% HCV/HIV coinfection in Southern Taiwan. However, it agrees with the

work of Okonko *et al.* [21] who found the prevalence rate of HCV antibody among fresh undergraduate students in Port Harcourt, Nigeria to be 0.0% and that of Opaleye *et al.* [22] who reported a 0.0% HIV/HCV coinfection prevalence among pregnant women in southwestern Nigeria. Also, studies by Alli *et al.* [23] also reported a 0.0% HCV seroprevalence rate in Ibadan, Oyo State.

The 0.0% reported in this study is also a contrast to the results of several studies on HIV/HCV coinfection prevalence conducted in different locations of the country. A 2.3% HCV seroprevalence was reported by Tremeau-Bravard *et al.*, [24] amongst HIV patients in Abuja. HIV/HCV coinfection prevalence among adult patients attending selected highly active anti-retroviral therapy clinics in Abuja was found to be 3.86% [25]. The 3.3% seroprevalence rate of HIV/HCV coinfection was reported by Akinbami *et al.* [26] in Lagos State. In Benin City, Ojide *et al.* [27] discovered a 7% HCV prevalence amongst adult HIV patients. Hamza *et al.* [28] also discovered a HCV prevalence of 1.6% among HIV-infected patients in a tertiary hospital in North-Western Nigeria.

Comparably low HIV/HCV coinfection prevalence has also been reported by certain studies. A 0.16% HIV/HCV coinfection prevalence was reported by Ikeako *et al.* [29] amongst antenatal women in a tertiary institution in south east, Nigeria. Omatola *et al.* [30] discovered a HIV/HCV coinfection prevalence of 0.5% among pregnant women in Anyigba, Nigeria. A 0.7% prevalence of hepatitis C virus among HIV patients in a suburban University Teaching Hospital also in South-East Nigeria has been reported by Diwe *et al.* [31]. A 0.8% HIV/HCV coinfection prevalence was discovered by Agboghroma and Ukaire [32] among pregnant women attending antenatal care at a tertiary hospital in Abuja.

HCV prevalence rates of 0.8% and 0.9% have also been reported by Oluremi *et al.* [33] and Onyekwere and Hameed [34] respectively. Despite these studies were not specifically conducted amongst HIV infected populations, it is a pointer that HIV/HCV coinfections is not common.

The variations which occur in HCV prevalence can be attributed to the characteristics of the population investigated such as the presence of susceptible age groups and risk factors for HCV

acquisition. These risk factors can also arise from the socio-cultural practices of the region investigated; for example, a HCV prevalence of 9.2% was reported in a study conducted in South-West Nigeria by Ogunro et al. [35] where over 90.8% of the participants had tribal marks. The prevailing socio-economic condition of a population can also act as a risk factor as a poor economy can pre-dispose its citizens to dangerous sexual practices, poor access to good healthcare and lack of education on HCV prevention strategies and good health practices. The sensitivity of the serological test used can also contribute to the variations as false positive and false negative test results have been known to occur.

4. CONCLUSION

This study has shown a zero prevalence of HIV and HCV coinfections. The zero HCV prevalence reported by this study should not evoke a relaxed attitude towards HCV infection in the city in view of the deleterious effects it has on the health of infected individuals. Despite the low rates of hepatitis coinfection observed in this study, routine screening for HBV and HCV markers should be carried out to reduce morbidity and mortality in this group.

CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this study. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the Research Ethics committees of University of Port Harcourt and have, therefore, been performed following the ethical standards laid down in the 1964 Declaration of Helsinki.

ACKNOWLEDGEMENTS

The authors are grateful to the management of the University of Port Harcourt Teaching Hospital (UPTH) for granting the approval required to perform this study and also the support rendered by her staff during the enrollment and collection of samples. The authors also appreciate all the

individuals who gave their consent and partook in the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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The peer review history for this paper can be accessed here:
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