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SEROLOGICAL PREVALENCE OF HCV/HIV CO-INFECTION AMONG HIV-INFECTED PATIENTS IN ONITSHA, ANAMBRA STATE, NIGERIA.

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ABSTRACT

Chronic hepatitis C (HCV) co-infection is prevalent in people with HIV (PLWHIV). This study investigated the relationship between the sociodemographic characteristics of HIV-infected patients attending the HIV clinic at a referral specialist mission hospital in Onitsha, Anambra State, Nigeria, and the potential for HCV co-infection. Two hundred and twenty (220) HIV-positive individuals consented to participate in the study. Blood samples (approximately 5 ml) were aseptically collected into sterile EDTA vials during routine investigations between March 2022 and April 2022, and plasma samples were obtained by centrifugation. Using the Partec CyFlow, CD4 counts were calculated. With the aid of Abbott Real-Time HIV-1 Assay US methodology, plasma viral loads (PVL) were also calculated. A total of 3 (1.4%) HIV-positive people were also confirmed to have HCV infection, where all the infected individuals were women (1.8%). Most of those affected were between 31 and 40 (2.9%). Concerning marital status, HCV co-infection was found more amongst separated/divorced patients, with a rate of 14.3%. The prevalence of HIV/HCV co-infection was higher (1.7%) in those with CD4 cell counts under >350 cells per ml. Most patients (1.9%) did not have their viral RNA detected (TND). Self-employed infected persons and those with tertiary educational backgrounds had the highest HIV/HCV prevalence rate of 0.7% and 2.4%, respectively. The study's findings show no statistically significant relationships between the patients' sociodemographic traits and HCV. Also, the prevalence of HCV co-infection was significantly low in the study participants.

1. INTRODUCTION

Two chronic viral infections affecting millions globally are hepatitis C (HCV) and HIV-1. According to the World Health Organization, approximately 71 million people globally were infected with HCV as of 2015, accounting for 1% of the global population (WHO, 2017).

Furthermore, it was estimated that 2.3 million (6.3%) of the 36.7 million people living with HIV in 2015 also had HCV (WHO, 2017). The possible reason for this large spread across the globe can be attributed to the viruses having similar modes of transmission by spreading via percutaneous contact with infected blood, sexual contact, and vertical transmission from infected mother to child (Gobran et al., 2021).

Studies have revealed that co-infection with HIV and hepatitis is becoming more prevalent in African populations. Sub-Saharan Africa has the highest burden of HCV in the world, with an estimated prevalence of 5.3% and a total of 32 million infected persons (Agboghoroma & Ukaire, 2020).

Nigeria, a country of roughly 200 million people, is known to have the world's second-highest HIV/AIDS prevalence, recently reported at 1.4% in 2019, an improvement from previous years. The prevalence of Hepatitis C virus (HCV) infection ranges from 2.8 to 24.2% among adults from different sub-regions of the country (Adesegun et al., 2020). several prevalence figures for hepatitis C viral infection have been reported in Nigeria across various study groups or populations. The prevalence rate is as follows. Approximately 2.1% of the general population, 12.3% of adult blood donors, 5% of sickle cell anaemia patients, and 11% of doctors and dentists (Omote et al., 2018). Several studies have also been done in different geopolitical zones on the prevalence of HCV co-infection in HIV patients (Nnakenyi et al., 2020). Prevalence of Hepatitis C co-infection rates of 14.6%, 4.7%, 23.3%, and 30.3% have been documented in the Northern, southeast, southwest, and North Central geopolitical regions of the country, respectively (Mabayoje, 2016; Odjimogho et al., 2018; Adesegun et al., 2020; Nnakenyi et al.,2020).

Identifying HIV/HCV co-infected patients is needed for proper management, as HCV infection is treatable (Yakubu et al., 2021). Given the scarcity of data on HCV/HIV co-infection, this research was embarked on to investigate the HCV co-infection in HIV-infected individuals attending an HIV clinic in a referral specialist hospital situated in Anambra state, southeast Nigeria

2. MATERIALS AND METHODS

2.1 Study Area

Sampling was done in a reference hospital that provides health care services to HIV-1 patients in Ànambra State, Nigeria; Saint Charles Borromeo Specialist Hospital, Onitsha, Anambra State, Nigeria.

2.2 Ethical standards

The study only proceeded after ethical approval was obtained from the Board of Research Ethics Committee for Saint Charles Borromeo Specialist Hospital, Onitsha, Anambra State, Nigeria. All consenting HIV-infected individuals attending the HIV clinics at these hospitals were considered eligible for recruitment in this study.

2.3 Study population

The study subjects were HIV-positive individuals attending the HIV clinic at Saint Charles Borromeo Specialist Hospital, Onitsha, Anambra State, Nigeria. Exclusion criteria were based on "no consent to participate' in the study. Those whose HIV status was unconfirmed and were seronegative were also excluded. Plasma samples (n=220) collected from March 2022 to April 2022 were analyzed in our study. Questionnaires with information including sociodemographics (age, sex, occupation, education levels, and marital status) were administered to the study participants, and trained interviewers collected the demographic data relevant to the study.

2.4 Sample collection/preparation

The venous blood samples were collected from the study subjects in the EDTA vacuum tube, and $CD4^+$ T-cell counts were measured within 24 h after sampling. Meanwhile, plasma samples were obtained by centrifugation and stored at −80 °C until further analysis.

2.5 Serological analysis

Serum antibodies against HCV were evaluated in vitro using an Enzyme-linked Immunosorbent Assay kit (DIA.PRO Diagnostic Bioprobes, Milano, Italy). The ma kit manufacturers' protocols regarding testing and result interpretation were followed. An ELISA plate reader was employed to read the optical signals produced in the microwells at 450 nm. The formula for calculating the cut-off OD450nm (OD of negative control plus 0.350) used as the threshold for differentiating between reactive and non-reactive serum samples was provided by the manufacturer of the ELISA kit.

2.6 Data Analysis

The data were recorded and analyzed using a Microsoft Excel spreadsheet (Microsoft Corporation). Data were analyzed using the Statistical Package for Social Science (SPSS) version 22.0. Chi-square or Fisher's exact was used where appropriate to test association. A p-value of

3. RESULTS.

The study featured two hundred and twenty (220) respondents that consented to participate in the research study. Males constituted 25.5% (n=56) of the study participants, while females comprised 74.5% (n=164). The sociodemographic characteristic of the respondents concerning their co-infection with HCV is presented in Table 1. HCV antibodies were detected in 3 (1.4%) of the 220 HIV-positive individuals, while 217 (98.6%) of the people were negative. The 3 (1.8%) HIV-positive persons that also tested positive for HCV antibodies were all females. 2.9%(n=2) and 1.4%(n=1) of those with HIV/HCV co-infection fell within the age group of 31-40 years and 41-50 years, respectively. The data from their educational status showed the highest HCV/HIV prevalence rate among those with tertiary-level registering a 2.4% rate, followed by primary and secondary levels of education, recording 1.8% and 0.8%, respectively. Those affected mainly were

separated and divorced in terms of their marital status, with a record 14.3% HCV/HIV prevalence rate, followed by the married (1.7%) among them. The study participants were all of the Christian religion that tested positive for HCV antibodies (1.4%). Only about 0.7% of the self-employed tested positive for HCV antibodies.

Table 1: Prevalence of Hepatitis C Antibody (HCV Ab) among HIV-positive individuals as it relates to their demographic characteristics

Variables	Number Tested	HCV +ve	%	Chi-Square test
Age groups (Years)				
Below 18	13	0	0.0	χ^2 = 2.254, p= 0.997
18-30	15	0	0.0	
31-40	70	2	2.9	
41-50	71	1	1.4	
Above 50	51	0	0.0	
Sex				
Males	56	0	0.0	χ^2 =0.572, p= 0.412
Females	164	3	1.8	
Educational Levels				
None	1	0	0.0	χ^2 = 0.871, p= 0.875
Primary	56	1	1.8	
Secondary	122	1	0.8	
University/Polytechnics	41	1	2.4	
Marital Status				
Married	121	2	1.7	χ^2 =10.037, p= 0.018
Single	37	0	0.0	
Separated/Divorced	7	1	14.3	
Widowed	55	0	0.0	
Occupational Status				
Student	2	0	0.0	
Self-employed	149	1	0.7	
Employed	36	0	0.0	
Unemployed	33	0	0.0	
Religion				
Christianity	216	3	1.4	χ^2 = 0.056, p= 0.946
Islam	0	0	0.0	

Traditional	4	0	0.0	
TOTAL	220	3	1.4	

The prevalence of Hepatitis C antibodies with respect to the virological and immunological markers of the HIV-infected individuals is shown in Table 2. Those HIV-positive persons (1.9%) co-infected with HCV had their viral loads not detected (ND), while those whose CD4 counts were greater than 350 had a prevalence rate of 1.7% HIV/HCV co-infection.

Table 2: Prevalence of Hepatitis C antibody with respect to the Virological and Immunological markers of the HIV-infected individuals in Borromeo Hospital, Onitsha, Anambra State.

Viral Load(Copies/ul)	No Tested	HCV +ve	%	Chi-Square test
<40	15	0	0.0	$\chi^2 = 0.412$, p= 0.981
40-1000	21	0	0.0	
>1000	23	0	0.0	
ND	161	3	1.9	
TOTAL	**220**	**3**	**1.4**	
CD4 (Cells/µl)	**No Tested**	**HCV +ve**	**%**	**Chi-Square test**
<200	17	0	0.0	$\chi^2 = 0.696$, p= 0.706
200-350	24	0	0.0	
>350	179	3	1.7	
TOTAL	**220**	**3**	**1.4**	

4. DISCUSSION

Hepatitis virus infection, particularly HBV and HCV, is often detected as a co-infection in HIV-positive patients, causing severe health challenges and potentially leading to death. Previous research has established that nearly 2.3 million individuals worldwide are co-infected with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) (Gobran et al., 2021), making it a severe threat to public health globally.

Thus this research was embarked upon to ascertain the seroprevalence of HIV/HCV co-infection among HIV-positive individuals attending the retroviral clinic in a referral hospital at Onitsha, Anambra State, Nigeria. In this investigation, three (1.4%) HIV-infected individuals were discovered to be co-infected with HCV, with the affected HIV-infected individuals being all females (1.3%). This agrees with reports from previous studies that people living with HIV (PLWHIV) and those co-infected with HCV were mostly females. (Ugwu et al., 2023; Okonko et

al., 2022; Munyemana, et al., 2021). However, a contrary report from Nepal was recorded by Shrestha et al. (2022), where the males with HIV were more likely to have HIV-HCV co-infection. The possible reason for this was attributed to sexual promiscuity in males.

Also, studies from Cameroon reported that women were the most affected by HIV infection, while men were affected mainly by HCV infection (Marceline et al., 2018). However, Saidu et al. (2020) recorded an even co-infection of HCV-Ab/HIV of 4(1.2%) between the male and female study participants in Benin City, Edo State, Nigeria. Records from other climes reveal a higher HCV/HIV incidence rate among male HIV patients. In Singapore, we have a 96.2% (Choy et al., 2019), 9.5% found in the mid-west region of Brazil (Freitas et al., .2014), 9.9 % in southern Brazil (Vecchi et al., 2022) and 83.3% in Egypt (Elrashdy e. al.,2022).

A review article published by Kenfack-Momo et al. (2022) revealed that the overall prevalence of HCV infection in the African continent in PLHIV was 5.4%. In our current study, the overall seroprevalence of HIV/HCV co-infection as low as 1.4 %(n=3) was observed. This co-infection is lower than the 4.0% reported by Okonko & Shaibu (2023) in Yenagoa, Bayelsa, Nigeria; lower than 22.5% in Port Harcourt (Okonko et al., 2022), 7.0% and 7.5% in Lagos, (Lawal et al., 2020), and 8.1% in Ondo State, (Ogundele et al., 2017) all in Nigeria. Additionally, Cookey et al. (2021) recorded a 0.0% co-infection rate in Rivers State, Nigeria. In Abuja, the capital city of Nigeria, Anyanwu et al. (2020) reported the prevalence of HIV/HCV co-infection to be 3.86%, amidst other co-infections such as HIV/HBV/HCV and HIV/HBV co-infections with 0.64% and 6.43% respectively. Furthermore, data from previous similar studies in other African countries have also been documented, such as in Kumasi Ghana; a 5.5% rate was recorded (Boateng et al., 2019), 13.3% seroprevalence of HIV/HCV co-infection was documented in Cameroon (Marceline et al., 2018). 12.5% in Rwanda (Munyemana et al., 2021) and 5.2% in Northeastern Ethiopia (Gedefie et al., 2021).

Concerning their various age groups, a 2.9 %(n=2) prevalence rate was the highest recorded among those in the age bracket 31-40 years, followed by those in the age bracket 41-50 years (1.4%). This tallies with the findings by Anyanwu et al. (2020), who recorded the highest HIV/HCV co-infection rate among patients aged 31–40 (8, 2.57%). A research study conducted in Nasarawa State, Nigeria, revealed similar findings where the highest HIV/HCV co-infection (2.5%;13.5% & 1.6%;11.5% recorded among study participants from Abuja and Keffi, respectively) fell within the same age groups of 31-40 years and 41-50 years. This result contradicts the reports of Opaleye et al. (2016), who found a higher prevalence of 25% in the age group 21-30 and 41-50 years. However, the findings of this study were still consistent with studies from other regions of the country that reported a higher prevalence of HCV among the same age groups, suggesting that this age group engages in more sexual activity. (Erasmus et al., 2021)

The study also revealed participants who had attained a tertiary level of education to be the most affected, recording a prevalence rate of 2.4%, followed by those with primary and secondary

education of 1.8% and 0.8%, respectively. This result corresponds to our findings from a recent study conducted in a tertiary hospital at Awka, Anambra State, Nigeria, where those with tertiary education levels showed the highest rate (8.6%) (Ugwu et al., 2023). Also, Agboghoroma & Ukaire (2020) reported a similar record of a 1.3% rate among pregnant women of tertiary education level. On the contrary, Akhtar et al., 2022 recorded the highest data of (8.5%) HIV/HCV co-infection among those with a primary education level and the least among graduates (0.3%). The reason for our outcome concerning the educational qualification of the study participants is yet to be ascertained since it is expected that exposure to higher learning/knowledge is considered a factor in the maintenance of personal hygiene.

Data in terms of their marital status established HCV/HIV prevalence rates of 14.3% and 1.7% for the divorced/separated and married patients, respectively. This observation partly agrees with other findings where HIV/HCV co-infection was detected more in married patients (Adesegun et al., 2020; Agboghoroma & Ukaire, 2020; Oluremi et al., 2020; Ugwu et al., 2023). Nonetheless, our data varied from results obtained from other studies. These findings contradict the result obtained by Seyoum et al. (2022) in Addis Ababa, Ethiopia, where the widowed showed the highest HCV co-infection, 2.50%, followed by never married (the singles), 2.38%, and the lowest was recorded among the married study participants (0.98%). In Malaysia, the highest record of 9.9% was found among singles, followed by the married with 6.2% (Akhtar et al., 2022).

Based on occupation and religious status, the HCV antibodies were detected only in the self-employed (0.7%) and Christian patients (1.4) %. The self-employment data also aligned with our findings in Awka Anambra State, Nigeria (Ugwu et al., 2023), as well as that reported by Anyanwu et al. (2020). Most of the study participants were of a Christian religious background, as that is the religion commonly practised in this region of the country; hence all those affected were Christians. However, Agboghoroma & Ukaire (2020) registered 1.9% of HCV/HIV co-infection among those practising Islam religion and 1.0% among Christians in Abuja. Religious affiliation is based on what is commonly practised in different geopolitical zones of the nation.

The HIV-infected individuals (1.9%) that were co-infected with HCV all had their viral loads not detected (ND), while those whose CD4 counts were greater than 350 had a prevalence rate of 1.7% HIV/HCV co-infection. Results obtained based on the virological marker indicator to the HIV/HCV co-infection are at variance with our findings from a recent similar study where the study participants with viral loads between 40 and 1000 copies/l had the highest prevalence of HIV/HCV (13.3%) (Ugwu et al., 2023). During the current investigation, study participants were supposedly under antiretroviral therapy, which should be a contributing factor to the viral loads reading undetected. Data concerning CD4 counts is similar to recent findings in which HIV/HCV co-infected study participants had a higher CD4 count (350-499 cells//l) (Okonko & Shaibu, 2023). Gedefie et al. (2021) reported that 5.8% of HIV/HCV co-infected individuals with CD4 counts greater than 200 in Northwest Ethiopia.

5. CONCLUSION

Conclusively, this study showed relatively low HCV antibodies (1.4%) detected among HIV patients attending an HIV clinic at a referral hospital**.** This observation validates the serological study of viral hepatitis in all HIV/AIDS patients. Emphasis should be laid on the diagnosis and appropriate management of viral hepatitis for people living with HIV/AIDS to lower mortality rates and lead to improved quality of life.

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