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Serosurvey of the Comorbidity of Hepatitis C Virus with HIV in Some Parts of Nasarawa State and Nigeria's Capital City: A Cross-Sectional Survey

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ABSTRACT

This research was undertaken to determine the seroprevalence of HCV among patients attending antiretroviral therapy (ART) clinics in some parts of Abuja and Nasarawa States, Nigeria. A total of 522 sera samples were collected between March and August 2019, from HIV patients, recruited in the ART clinics of selected hospitals. Samples were screened using immunochromatographic assay, followed by a third-generation Enzyme-Linked Immunosorbent Assay (ELISA). 154 (29.5%) of these samples were from male patients, and 368 (70.5%), females with a ratio of 1:2.4. The mean age (± SD) of the patients was 38.0 (± 9.56). The presence of hepatitis C virus co-infection was observed in 82 (15.7%) patients. The male population had a higher co-infection rate (17.5%) than the female population (14.9%). Patients aged 31-40 had a higher hepatitis co-infection (6.7%) rate than the other age groups. Blood transfusion, sharing of sharp objects, and the number of sex partners were significantly associated with HCV co-infection. This study revealed high endemicity of hepatitis C virus co-infection with HIV in the study region. Preventive measures are thus advocated to curb the spread of the viral infection.

Keywords: Hepatitis C virus, HIV, Comorbidity, Nigeria, Transmission-Transmissible Infection (TTI), Seroprevalence.

Abbreviations: HCV: Hepatitis C Virus; ART: Antiretroviral Therapy; HIV: Human Immunodeficiency Virus; ELISA: Enzyme-Linked Immunosorbent Assay; TTI: Transfusion-Transmissible Infection; HAART: Highly Active Antiretroviral Therapy; FCT: Federal Capital Territory; RC: Research Centre; SPSS: Statistical Package for Social Sciences; SD: Standard Deviation; RNA: Ribonucleic Acid

INTRODUCTION

Hepatitis C virus belongs to the family Flaviviridae under genus Flavivirus. It is a spherical enveloped single-stranded RNA virus. Blood transfusion, non-sterile needles, sexual intercourse, nosocomial patient-to-patient transmission (which may occur through the contaminated colonoscope, via dialysis or during surgery like organ transplant), and mother-to-child transmission are modes of transmission of Hepatitis C virus [1]. Symptoms of Hepatitis C virus (HCV) include arthralgias, sicca syndrome, sensory neuropathy, variceal bleeding, etc. Hepatitis C virus co-infection with human immune deficiency virus has become a major cause of morbidity and mortality [2].

Worldwide it is stated that approximately 71 million persons are chronically infected with HCV. About 4-5 million persons of the population clinically infected with HCV are co-infected with HIV [3]. HCV has been classified into six genotypes [1-6], with each genotype further subdivided into subtypes showing different geographical

distributions worldwide [4]. In sub-Saharan Africa, Hepatitis C virus co-infection with human immunodeficiency virus is endemic [5].

In Nigeria, the prevalence of HIV co-infection with HCV varies with sub-region. Their co-prevalence has been reported sporadically in different sub-populations. In the South-western part of the country, HIV/HCV co-infection rates ranging from 1.5% to 23.2% have been reported [6-12]. Lower rates (<10%) have been recorded in other Southern parts of Nigeria [13-16]. The co-infection rates of 8.2% and 2.3% have previously been reported in Abuja [17-19], while the comorbidity rate in Keffi was previously reported to be above 10% more than a decade ago [20]. In other North-central parts of the country, previous reports of HIV/HCV range from 4.7 to 11.3% [3,20-22]. Hepatitis C virus infection is a major public health challenge, especially in nations that are less developed where treatment is either inaccessible or expensive. It occurs among people of all ages, genders, races, and world regions. Nigeria is recognized to be highly endemic for viral hepatitis. It is estimated that one-third of HIV positive individuals are co-infected with HCV worldwide [13,23].

The diagnostic algorithms employed for the routine screening of HCV vary with location. Screening tests include immunochromatographic assays, Enzyme-Linked Immunosorbent Assays (ELISA), Nucleic Acid Amplification Tests (NAAT) and Recombinant Immunoblot Assay (RIBA). The fact that Human Immunodeficiency Virus (HIV) positive patients stand the greater risk of being affected with many diseases including hepatitis C in endemic regions like Nigeria, initiated this study to ascertain the prevalence of Human Immunodeficiency Virus (HIV) patients co-infected with Hepatitis C Virus (HCV) in some parts of Nasarawa State and the Federal Capital Territory using serological analyses.

MATERIALS AND METHODS

Research design, area, and population

This cross-sectional study randomly captured patients aged 18-70 years attending the ART clinics in Garki Hospital Abuja and Maitama District Hospital, Federal Capital Territory (FCT), and Federal Medical Center Keffi, Nasarawa State, Nigeria. The cohorts consisted of patients that were confirmed to be HIV-positive and already commenced ART. On each clinic day, ten adult patients who were confirmed to be HIV-positive were randomly selected to participate after being informed about the study. Patients who declined to participate were replaced by other random participants.

Ethical approval and informed consent

Ethical approvals were obtained from the Health and Research Ethics Committee governing the hospitals: Garki Hospital Abuja (FHREC/2019/01/11/18-02-19), Maitama District Hospital (FCTA/HHSS/HMB/GEN/O38/T) and Federal Medical Center, Keffi (Research Ethics Committee of Nasarawa State Ministry of Health (NHREC/ 21/02/2019)). Informed consent forms were signed by the included patients.

Inclusion and exclusion criteria

Individuals in the target study locations who were confirmed to be HIV-positive and using the services of the ART clinics at the time of the study, who fell under the ages of 18-70 and gave their consent, were included. All included patients were either oblivious of their HCV status or had previously tested negative to HCV. HIV seronegative individuals were excluded from this study. HIV positive patients who did not give their consent; those below 18 years of age, and those who indicated interest after the maximum number of samples had been collected were also excluded. Patients who tested positive to HCV in the past were excluded.

Sample processing and collection

A total of 522 blood samples from HIV seropositive patients were obtained between March and August 2019 for this study. The samples were collected by the phlebotomist through vein puncture of the antecubital fossa, after swabbing the area. Structured questionnaires were used to obtain socio-demographic information and patients' responses to predisposing factors. Samples were collected in sterile well-labeled plain vacutainer tubes and immediately transported to Zankli Research Centre (RC), Bingham University, Karu, for further processing.

At Zankli RC, the blood samples were centrifuged at 2200-2500 revolution per minute (rpm) for 15 minutes to separate the serum. The sera were aliquoted into 2 ml cryovials and stored at -20°C, after which the sera were

screened for hepatitis C virus antibodies using HCAb OnSite Rapid Test Cassettes (CTK Biotech, Inc., CA 92064, USA) based on double antigen lateral flow chromatographic immunoassay. Two red lines on the test (T) and control (C) regions indicated a positive result, while a single line on the C region indicated a negative result. Seropositive samples were further analysed with Enzyme-Linked Immunosorbent Assay (ELISA) using a third-generation HCV rapid ELISA kit (Diagnostic Automation Inc, USA), based on solid-phase indirect ELISA principle, following the manufacturer's instructions. Sera samples with an Optical Density (OD) to cut off (C.O) ratio ≥ 1.0 at 450 nm were considered positive for HBV infection; OD/C.O <1 were regarded as negative results, while borderline results fell between 0.9-1.1. The Rapid Diagnostic Test (RDT) was backed up by ELISA to detect possible cases of false-positive tests since RDT is used for routine screening in the hospitals, third-generation ELISA which has >99% sensitivity and specificity was employed. ELISA was done in duplicates for quality assurance.

Statistical analysis

Data collected were subjected to descriptive analysis involving the generation of frequency and mean, standard deviations, or proportions for continuous or categorical distribution of different variables. Pearson's Chi-square analysis was used to test all socio-demographic data and relationships between variables using SPSS version 25 (IBM SPSS Incorporated, Chicago, IL, USA). Pearson's bivariate correlation (R) was employed to evaluate the association between HIV/HCV seroprevalence and predisposing factors. Generated data were presented in tables and figures. P-values were considered statistically significant at <0.05.

RESULTS

A total of 522 samples from HIV seropositive patients aged 18-70 years were screened for Hepatitis C Virus (HCV) in this study, 154 (29.5%) of whom were male and 368 (70.5%), female. The male to female ratio was 1:2.4. The mean age (± SD) was 38.0 (± 9.56). Eighty-two patients (15.7%) were co-infected with HCV (Figure 1). Co-infection rates of 5.9% (19/322) and 31.5% (63/200) were obtained from Abuja (Nigeria's capital city) and Keffi (Nasarawa state), respectively.

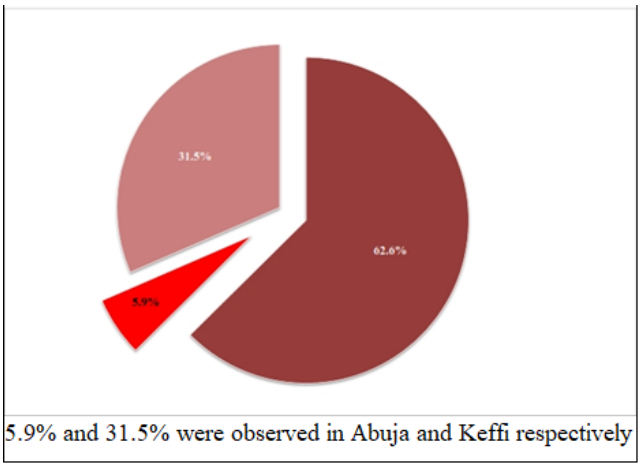

Figure 1: Percentage seroprevalence of HCV infection among HIV positive patients in Abuja and Keffi

Out of the males and females examined, the comorbidity rate of HCV with HIV within gender was 17.5% (27/154) and 14.9% (55/368) for male and female patients, respectively (p=0.459). This shows a higher prevalence in male patients than female patients (χ^2=0.549, df=1, p=0.459). The percentage seroprevalence of HIV/HCV co-infection across gender for each study location is presented in Figure 2. There was no significant difference between seroprevalence and the gender of the patients (Abuja: χ^2=0.392, df=1, p=0.531; Keffi: χ^2=0.640, df=1, p=0.424)

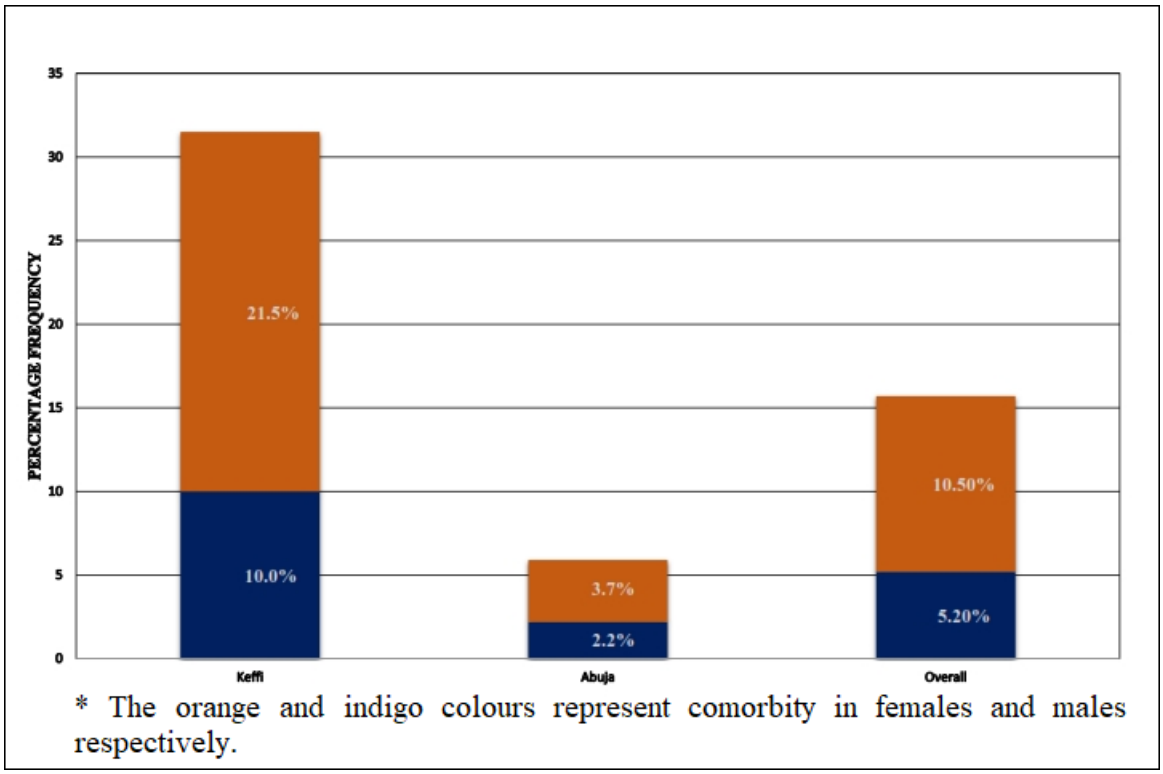

Figure 2: HCV co-infection rate with HIV in accordance to gender of study participants

The percentage comorbidity rate of HIV/HCV according to age within the study population is seen in Table 1. The highest co-infection rate was seen in the age group 31-40 (6.7%), and the least was observed among patients below 20 years (0%). The highest co-infection rates in the two study locations were also observed among patients aged 31-40 (2.5% and 13.5%) in Abuja and Keffi, respectively. No significant difference was observed in the association between age group and the prevalence of HCV antibodies (χ^2=4.665, df=5, p=0.458).

Table 1: Distribution of HIV/HCV co-infection among the patients according to age

Age group (in years)	Abuja		Keffi		Overall	
	Number examined	Number positive (%)	Number examined	Number positive (%)	Number examined	Number positive (%)
<20	4	0 (0.0)	0	0 (0.0)	4	0 (0.0)
20-30	44	2 (0.6)	22	5 (2.5)	66	7 (1.3)
31-40	144	8 (2.5)	90	27 (13.5)	234	35 (6.7)
41-50	106	5 (1.6)	58	23 (11.5)	164	28 (5.4)
51-60	22	4 (1.2)	22	5 (2.5)	44	9 (1.7)
61-70	2	0 (0.0)	8	3 (1.5)	10	3 (0.6)
Total	322	19 (5.9)	200	63 (31.5)	522	82 (15.71)

Abuja: χ^2=6.796, df=5, p=0.236; **Keffi:** χ^2=3.584, df=5, p=0.465; **Overall:** χ^2=4.665, df=5, p=0.458. NB: Percentages were calculated from total number of participants in each study area, and overall percentages were calculated from the sum total of participants (i.e., number Examined)

Among the participants, 106 were single, 52 were widowed, 350 were married, and 14 were divorced. The percentage of HCV co-infection with HIV according to marital status in the sampled population is shown in Table 2. The highest occurrence was found among married patients (10.3%, 54 positive cases), while two cases of hepatitis C

virus co-infection was found in divorced patients (0.4%). A similar trend was observed in the two different locations with the highest rates of 4.00% and 20.50% among married patients from Abuja and Keffi, respectively (p>0.05).

Table 2: Distribution of HCV co-infection with HIV in relation to marital status of the patients

Marital Status	Abuja		Keffi		Overall	
	Number examined	Number positive (%)	Number examined	Number positive (%)	Number examined	Number positive (%)
Married	218	13 (4.00)	132	41 (20.50)	350	54 (10.30)
Singles	70	3 (0.90)	36	9 (4.50)	106	12 (2.30)
Widowed	22	2 (0.60)	30	12 (6.0)	52	14 (2.70)
Divorced	12	1 (0.30)	2	1 (0.5)	14	2 (0.4)
Total	322	19 (5.90)	200	63 (31.50)	522	82 (15.71)
Overall: $\chi2$ =6.522, df=3, p=0.089; **Abuja:** $\chi2$ =0.861, df=3; p=0.835; **Keffi:** $\chi2$ =2.038, df=3, p=0.564						

A total of 125 of the participants were unemployed, 229 were self-employed, while 168 were employed. Antibodies to Hepatitis C virus co-infection were highest among the self-employed patients (6.70%) and the lowest among the unemployed patients (4.00%), as shown in Table 3. Patients who had primary education were also significantly the most co-infected with HCV with a rate of 6.70% (p<0.05). HIV/HCV seroprevalence was significantly higher among the rural dwellers than the urban settlers (p=0.000; Table 3).

Table 3: Distribution of HIV/HCV co-infection in relation to the Place of living, educational and employment profile of ART patients

Variable	Abuja				Keffi				Overall			
	Number examined	Number positive (%)	$\chi2$	P value	Number examined	Number positive (%)	$\chi2$	P value	Number examined	Number positive (%)	$\chi2$	p-value
Place of living												
Rural	195	16 (5.00)	4.729	0.030*	182	58 (29.00)	0.127	0.722	377	74 (14.20)	15.749	0.000*
Urban	127	3 (0.90)			18	5 (2.50)			145	8 (1.50)		
Total	322	19 (5.90)			200	63 (31.50)			522	82 (15.71)		
Educational Qualification												
None	10	0 (0.00)	2.871	0.412	20	1 (0.50)	7.797	0.050	30	1 (0.20)	17.960	0.000*
Primary	30	0 (0.00)			102	35 (17.50)			132	35 (6.70)		
Secondary	136	9 (2.80)			56	18 (9.00)			192	27 (5.20)		
Tertiary	146	10 (3.10)			22	9 (4.50)			168	19 (3.60)		
Total	322	19 (5.90)			200	63 (31.50)			522	82 (15.71)		
Employment Status												
Unemployed	65	3 (0.90)	0.388	0.824	60	18 (9.00)	3.713	0.156	125	21 (4.00)	0.151	0.928
Self Employed	133	9 (2.80)			96	26 (13.00)			229	35 (6.70)		

Employed	124	7 (2.20)			44	19 (9.50)			168	26 (5.00)		
Total	322	19 (5.90)			200	63 (31.50)			522	82 (15.71)		
*Distribution of HIV/HCV is significant at the p=0.000												

The frequencies of occurrence, as well as the relationship between HIV/HCV comorbidity and the predisposing factors, are presented in Table 4. While the other risk factors showed no significant correlation, blood transfusion (i.e., reception), re-use/sharing of sharp objects, and the number of sex partners were significantly associated with HIV/HCV co-infection in this study ($p<0.05$).

Table 4: Pearson's Bivariate Correlation between HIV/HCV co-infection and associated risk factors among ART patients

Variable	Response	Number examined N=522	Frequency (%)	Correlation (R)	p-value
Blood donation	Yes	72	10 (1.90)	-0.020	0.634
	No	450	72 (13.80)		
Number of times	0	450	72 (13.80)	-0.006	0.900
	1	32	3 (0.60)		
	>1	40	7 (1.30)		
Number of sex partners	0	56	7 (1.30)	0.098	0.025*
	1	355	49 (9.40)		
	>1	111	26 (5.00)		
Use of condoms	Yes	284	43 (8.20)	-0.017	0.698
	No	238	39 (7.50)		
Unprotected sex	Yes	462	75 (14.40)	0.040	0.361
	No	60	7 (1.30)		
Re-use/Sharing of Sharp objects	Yes	196	40 (7.70)	0.100	0.022*
	No	326	42 (8.00)		
Blood transfusion	Yes	78	29 (5.60)	0.247	0.000**
	No	444	53 (10.20)		
Key: **Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed)					

DISCUSSION

Viral hepatitis is a leading cause of liver-related death among patients with HIV/AIDS worldwide, and HCV is one of the aetiological agents. This study examined the co-infection rate of Hepatitis C virus in HIV seropositive patients attending ART clinics in parts of Abuja and Nasarawa state by screening 522 HIV seropositive samples using anti-HCV immunochromatography and ELISA assays. The male to female ratio in this study was 1:2.4. The mean age (± SD) of the participants in this study was 39.0 (± 8.27), and 82 patients (15.71%) had antibodies to HCV, indicating co-infection with HIV.

The observed prevalence was 15.71%-31.50% in Keffi, Nasarawa State, and 5.90% in Abuja, Nigeria's capital city. The 15.7% co-infection rate recorded in this study is higher than the findings from previous researchers in these study locations, as well as different parts of the country-Keffi, 11% [20]; Abuja, 2.33% [19]; Suleja, 4.7% [3]; Jos, 5.7% [21]; Ibadan, 4.8% by [24]; Enugu, 10% [14]; and Makurdi, 2.3% [25]. It is also higher than the 7.8% observed

in Thailand [26], as well as the zero prevalence reported in Cote d'Ivoire and Kenya [27,28]. However, it is lower than the report of Mabayoje and co-authors [9], who obtained a prevalence of 23.2%. It should particularly be noted that findings from this study vary in increased proportion from previous reports obtained in the same region. This finding is suggestive of an increase in the endemicity of HCV co-infection with HIV in the region. This finding could be linked to increased exposure to several risk factors associated with the infection. The higher rate of 31.50% observed in Keffi is however not surprising, as it's a town with a suburban setting, compared to the metropolitan structure of Abuja, the capital city of Nigeria.

From the total population studied, more females (10.50%) than males (5.20%) had antibodies to HIV/HCV co-infection. However, within gender, more males (17.50%) than females (14.90%) were co-infected with HCV. Although the trends are similar within gender, this report is extremely lower than that of Mabayoje and co-authors [9], who obtained higher prevalence in males (60%) and females (40%), and the 56.1% and 43.9% that were reported among males and females [29]. The finding is higher than the 7.5% among males and 4.5% among females [21]; 1.4% among males and 0.4% among females [19]; 12.7% among males and 2.1% among females [30]. Findings across gender align with the following reports: [15] who found a prevalence of 7.1% in females and 6.7% in males and [11], who obtained 1.90% in females and 0.63% in males. The conflicting difference in sex-based seroprevalence compared to previous studies may be due to epidemiological differences in the different study populations and variations in methodology. The higher prevalence obtained within the male gender in this study might be due to social and sexual practices such as intravenous drug use, multiple sex partners, and unprotected sex, which tends to be more preponderant among males. Also, the level of estrogen in females is said to play a vital role in HCV clearance.

The highest frequency of HCV co-infection with HIV was seen among patients aged 31-40 years (6.70%). This contradicts the reports of Opaleye, et al. [11], who found a higher prevalence of 25% in the age group 21-30 and 41-50 years. The difference between the age groups studied was not statistically significant ($p>0.05\%$). Younger generations are more at risk of Hepatitis C virus infection due to their higher tendencies to being predisposed to risk factors, especially via their sexual and social inclinations. The age-specific distribution of HIV/HCV co-infection in this study showed no steady increase or decline with age, further indicating that age was not an important factor for HCV co-infection with HIV.

Participants that were married had the highest prevalence of 10.30%. This finding corresponds with the reports of Mabayoje and co-authors [9], who reported the highest incidence (66.1%) in married participants and investigation Oshun and Odeghe [31], who documented 87.4% in married participants. Our findings, however, disputes the report by Olanisun and co-authors [32], who found a higher prevalence among widowed individuals. This could be attributed to sexual practices, possible cases of infidelity, including an increased rate of unprotected sexual practices among this group. Som`e of these patients harbor the mindset that no STI could be worse than the HIV for which they are already undergoing treatment, and may tend to be spontaneous or unconscious of mandatory protection during sex.

Co-infection serosurvey based on place of living, employment, and educational profile of the patients revealed the highest comorbidity rates to be among rural dwellers (14.20%), primary school leavers (6.70%) and self-employed individuals (6.70%). It should be noted that the highest rate obtained among primary school holders was observed solely in Keffi, as against other subgroups whose rates were shared between the two locations. There was an ominous gap between the co-infection rate in primary school leavers and other subgroups in Keffi. Firstly, in Keffi, most of the patients sampled had a primary level of education (n=102). Furthermore, there is a direct proportionality between socio-economic status and the dissemination of infectious diseases in developing countries, and the place of residence, level of education, and employment status are indicators of the economic profile of the developing world. Hence, the high rate observed among primary school leavers is not surprising, giving the poor educational background. The rural dwelling also has an indirect link with the distribution of hepatitis viruses due to the minimal emphasis placed on hygiene and the overall wellbeing of the inhabitants.

Pearson's bivariate correlation analysis of the relationship between HCV co-infection with HIV and predisposing factors revealed a significant association between blood transfusion (i.e., reception, $p=0.000$), use of sharp objects (0.022), as well as the number of sexual partners (0.025). Positive correlations were observed between these three factors and HIV/HCV seroprevalence. The other risk factors showed no significant effect. Most medical laboratories in Abuja now engage in mandatory anti-HCV antibody screening on all healthy donors before transfusion, hence, seropositive donors are not allowed to donate blood; blood and blood products are also routinely screening before transfusion. This standard may not always be obtainable in Keffi where, due to limited resources, there might not be strict adherence to standard practices of screening potential blood donors for transfusion-transmissible infections

before donation. Transfusion-transmission might also have been successful during the pre-seroconservation window period or occult viral hepatitis infection widely known by the absence of detectable levels of anti-HCV antibodies, as well as extremely low level of nucleic acid.

Since all the study participants captured in Abuja had prior knowledge of HCV infection, this variable was only surveyed among participants from Keffi. We observed a high rate of HCV co-infection with HIV (25.50%) among patients who did not know HCV or the infection, although this finding was not statistically significant ($p>0.05$). This depicts that prior knowledge of HCV infection is an important contributive factor in the transmission of the virus. Having a history of organ transplants was also found to be significantly associated with HCV co-infection as 50% of the patients who had a history were co-infected with HCV ($p=0.011$). Other risk factors such as drug use, tattoo piercing, previous haemodialysis treatment, family history of liver cancer and/or liver disease were not examined in the Keffi population because they all responded negatively to the questions, thus, leading to colinearity in data analysis. However, these risk factors showed no significant association with HCV co-infection among the Abuja residents ($p>0.05$).

CONCLUSION

The result obtained from this study revealed the comorbidity of HCV with HIV to be 15.71%, which implies that hepatitis C virus infection is endemic among HIV patients in the two study locations. The higher rates obtained in the different locations when compared to previous findings from those regions are also a cause for concern as the increased endemicity poses a threat, not just to HIV positive patients, but also to healthy individuals. The higher co-infection rate observed in Keffi highlights the need for preventive measures to curb the spread of the virus. Transfusion-transmission was implicated in this study, which is mostly obtainable via commercial blood donation. Lack of awareness is also an important contributing factor to HCV transmission. HIV co-infection with HCV affects the prognosis of HIV disease; hence, it has become important to initiate strategies for instant mediation due to its public health significance.

RECOMMENDATIONS

The following recommendation can be emphasized to serve as preventive and control measures for the spread of the Hepatitis C virus in the study area and the general public at large: Programs such as health education should be employed to educate or enlighten inhabitants of small towns and suburbs on the dangers of Hepatitis C virus infection and factors that could predispose them to the infection. Potential blood donors should undergo mandatory screening to ascertain their HCV infection status before blood transfusion. HIV patients should be routinely screened for HCV infection before their commencement of ART and in the course of therapy, to better guide physicians on the best therapeutic option. Population study is advocated to determine the prevalence of HCV and its co-infections in the general population.

LIMITATIONS

Due to limited resources and unavailability of the required facilities, we could neither go further to carry out HCV RNA testing nor identify the genotypes of the HCV detected in this study. The credibility of the study was therefore ensured by the use of the third-generation ELISA assay which has at least 97% sensitivity [33]. Furthermore, the study was hospital-based; thus, even though it gives a hint of what may be obtainable in the general population; conclusive inferences cannot be drawn for the general population. The study was also a cross-sectional survey, and so, any existing causal relationship between the period of exposure and resulting infection were not established.

ETHICAL APPROVAL AND INFORMED CONSENT

Written informed consent was obtained from the participating patients; and ethical clearance was obtained from the Health Research Ethics Committee of the Federal Capital Territory, Nigeria (FHREC/ 2019/01/11/18 02 19), Maitama District Hospital (FCTA/HHSS/HMB/GEN/038/T) and the Research Ethics Committee of Nasarawa State Ministry of Health (NHREC/21/02/2019). Written Informed consent to participate in the study was obtained from the study participants before their being enrolled consecutively.

CONSENT FOR PUBLICATION

Not applicable

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