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Hepatitis C And E Seromarkers Among Drug Experienced HIV Patients Attending Federal Medical Centre, Keffi, Nasarawa State, Nigeria

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Abstract: Human Immunodeficiency Virus (HIV), Hepatitis E Virus (HEV), and Hepatitis C Virus (HCV) are significant public health challenges worldwide, particularly in Sub-Saharan Africa. Co-infection of HIV with HEV and/or HCV is common due to shared transmission modes, leading to increased morbidity and mortality. A cross-sectional study was conducted at Federal Medical Centre, Keffi, Nigeria, involving 289 HIV-seropositive adults. Serum samples were tested for HEV and HCV markers using ELISA and PCR. Sociodemographic and clinical data were collected using a structured questionnaire. Statistical analysis was performed using SPSS. The prevalence of HEV-HIV co-infection was 12.8% (37/289), HCV-HIV co-infection was 6.9% (20/289), and HIV-HEV-HCV co-infection was 9.3% (27/289). HEV infection was significantly associated with gender ($p = 0.002$) and age ($p = 0.031$). HCV infection was associated with CD4+ T cell count ($p = 0.018$) and HIV viral load ($p = 0.022$). Co-infection was associated with increased HIV viral load and decreased CD4+ T cell count. The high burden of HEV and HCV co-infections among HIV-seropositive individuals highlights the need for routine screening and treatment to reduce morbidity and mortality. Strengthening healthcare systems, increasing access to diagnosis and treatment, and promoting awareness are essential to reducing the impact of these co-infections.

***Keywords*:** Hepatitis E Virus (HEV), Hepatitis C Virus (HCV), HIV co-infection, Seroprevalence, Nigeria

I. INTRODUCTION

The blood borne pathogens, Human Immunodeficiency Virus (HIV), Hepatitis E Virus (HEV), and Hepatitis C Virus (HCV) are still a significant worldwide public health challenge (Joint United Nations Programme on HIV/AIDS, 2017; World Health Organization, 2017). Globally, it is estimated that almost 37 million people are infected or living with HIV and approximately two thirds of the infected individuals live in Sub-Saharan Africa (Joint United Nations Programme on HIV/AIDS, 2017). More than 250 million and 70 million people are also estimated to be chronically infected with HEV and HCV, respectively (World Health Organization, 2017).

The most common cause of hepatitis worldwide are viruses (viral hepatitis). Other causes include consumption of heavy alcohol, certain medication, toxins, other infections and autoimmune diseases (National Institute of Diabetes and Digestive and Kidney Diseases, 2014; National Institute of Allergy and Infectious Diseases, 2016; World Health Organization, 2016). Both HEV and HCV are hepatotrophic viruses that account for 96% of all hepatitis mortality. They cause severe morbidity including cirrhosis and hepatocellular carcinoma (HCC) due to intra-hepatic apoptosis and mortality particularly among HIV-infected individuals (World Health Organization, 2017).

The presence of HBsAg in the blood is the specific serologic marker for HEV infection and there are a number of sensitive enzyme immunoassays (EIA) that have improved the detection of HEV. However, it was recognized that there may be individuals with acute and chronic Hepatitis E infection and asymptomatic patients where HBsAg levels may be too low to detect with EIAs (Satoh et al., 2008).

The introduction of antiretroviral therapy (ART) has significantly decreased HIV/AIDS-related morbidity and mortality (Noubiap et al., 2015). However, deaths resulting from non-AIDS-related illnesses have been on the increase. Worldwide, a large number of people living with HIV (PLHIV) die from non-AIDS illnesses including liver diseases (Farahani, 2017). The World Health Organization (WHO) recommends that all PLHIV including children regardless of their CD4 counts should initiate ART (World Health Organization, 2016).

Co-infection of HIV with viral hepatitis (HEV and/or HCV) occurs commonly because these viral infections share similar mode of transmission (Ferreira, 2016). HEV has ten genotypes (A–J), which have distinct geographical distribution (Sousa et al., 2018). HCV has six main genotypes (1–6) with multiple subtypes. Genotyping is most significant for planning of HCV treatment and helps to cure HCV infections (Islam et al., 2015).

In Nigeria, HIV-infected patients are not routinely screened for hepatitis viruses. Screening for HEV and HCV is only considered following observed deranged liver enzymes. Thus, there is no room for early detection of co-infections and institution of proper management of cases. Again, data on the prevalence of co-infection in our environment are still relatively scarce.

The high burden of HEV and HCV co-infections among PLHIV necessitates routine screening and treatment. Strengthening healthcare systems, increasing access to diagnosis and treatment, and promoting awareness are essential to reducing the impact of these co-infections.

II. MATERIALS AND METHODS

2.1 Study Area

The study was conducted at Keffi while the sample area is Federal Medical Centre, and located in Nasarawa State, North-Central Nigeria. Keffi is bounded by Karu LGA to the north, Akwanga LGA to the south, Lafia LGA to the east, and Abuja, FCT, to the west. Keffi's latitude is 8°50'N, and longitude is 7°52'E, with an elevation of 300 meters above sea level.

The population of Keffi is approximately 92,664 people (NPC, 2006), predominantly Afo, Eggon, Mada, Hausa, and Gwandara tribes. The major occupation is farming, with crops like maize, millet, and sorghum being cultivated. The people are predominantly Muslims and Christians, with a mix of traditional cultural practices.

2.2 Study Population

The study subjects included adult's population of both gender from within Federal Medical Center Keffi who were accessing medical care.

2.3 Sample Size

The sample size for this study was determined using the formula as reported by Naing*et al*. (2006).

$n = z^2 [pq] / d^2$

Where n= minimum sample size

z=standard error [1.96] at 95% confidence limit

p=local prevalence of 38.2% % or 0.382 (Junaid *et al.*, 2014).

q = [1.0-p]

d = degree of accuracy of 5% or 0.05

$n = (1.96)^2[0.382 \times (1.0-0.382)]/0.05^2$

$n = z^2 [pq]/d^2$

$n = [1.96]^2 \times 0.382 \times 0.758/[0.05]^2$

n = 3.8416 x 0.183436/0.0025

n = 0.704688/0.0025

n = 279

Attrition rate of 10% = 10

Total minimum sample to be collected = 289.

The total minimum number of samples to be collected was 402, however, 402 samples were collected to minimize error.

2.4 Study Design

The study was a cross-sectional study involving both male and female in adult population. Each participant's consent was sought and socio demographic and clinical information was obtained by means of a structural questionnaire

2.5 Ethical Consideration

Ethical clearance to conduct this study was sought from the Ethical Review Board/Ethical Committee of Federal Medical Center Keffi, Nasarawa State.

2.6 Sample Collection

The chemical pathology unit of the laboratory was used as the collection point. A total of 289 blood samples were collected from consenting patients. The samples were collected by venepuncture whereby the arm of the individual was tied with a tourniquet and the position of any prominent vein was disinfected using cotton wool soaked in methylated spirit. Using a disposable sterile needle and 5ml syringe for each participant, 3ml of blood sample was collected and transferred into a labelled plastic micro-litre tube (Aggarwal2012).

2.7 Sample Processing

To obtain serum, each blood sample was allowed to stand for some time and centrifuged at 3000rpm (revolution per minute) for 10 minutes at 37^{o}C. The sera was harvested and transferred into labelledcryovial tubes for storage. All samples were stored at minus 15^{o}C in the Department of microbiology laboratory Nasarawa State University Keffi freezer until ready for use.

2.8 Laboratory Analysis

2.8.1 Screening for Anti-HEV

All serum samples were tested using hepatitis E serological markers (anti-HEV IgG) (recomLineIgG, MikrogenGmBH, Germany) according to the manufacturer's instructions It is a rapid test kit for the detection of HEV in human serum. Positive samples were confirmed using recomScan test strip analysis software (recomScan computer software; Mikrogen GmbH) (Aggarwal, 2012).

2.8.1.1 Test Procedure

The HEV rapid test (recomLineIgG, MikrogenGmBH, Germany) is a qualitative, membrane-based immunoassay for the detection of antibodies to HEV in serum. The membrane is pre-coated with recombinant HEV antigen on the test line region of the cassette. During testing the specimen reacts with recombinant HEV antigen conjugate colloid gold. The mixture migrates vertically on the membrane chromatographically by capillary action to react with recombinant HEV antigen on the membrane and generate a coloured line. Presence of coloured line indicates a positive result, while its absence indicates a negative result. To serve as a procedural control a coloured line will always appear at the control line region.

The stored specimen and the test kits were first brought to room temperature, after which the foil wrapped pouch was opened and the cassette removed. Holding the pipette vertically a drop of serum was placed into the sample well and two drops of buffer was then added and results read after 10 minutes.

2.8.2 Screening for Anti-HEV using ELISA

To detect anti-HEV total antibodies, samples were analysed using the MP diagnostics HEV ELISA 4.0 (MP Biomedicals Asia Pacific, Singapore). Samples positive for anti-HEV total antibodies were further screened for acute infection using the MP diagnostics HEV IgM ELISA 3.0 (MP Biomedicals Asia Pacific, Singapore).

2.8.2.1 Determination of HEV Genotypes

A two-step nested reverse-transcriptase polymerase chain reaction (RT-PCR) for HEV detection and genotyping (Saha, *et al.*, 2014 &Green, & Sambrook, 2019) was used.

2.8.2.2 HCV RNA Extraction

RNA Extraction was carried out using Bionner extraction machine. Five Eppendorf tubes were labelled, corresponding with the samples for extraction and 500µl of lysis buffer was added into the tubes. The different samples for extraction were vortexed and 200µl of the samples was added to the corresponding labelledEppendorf tubes, then mixed by pipetting and vortexed. Aliquots of 150µl of Elusion buffer was placed into new Eppendorf tubes and incubated at 37°C in the heat block for 20 minutes.

The samples were centrifuged at 11,000 rpm to remove bubbles in the tubes and further transferred into the binding column, placed on ice pack and centrifuged at 12,000 rpm for one minute. The flow through was removed and discarded and 600 µl of wash buffer was added and then centrifuged at 12,000 rpm for 1minute. The flow through was then discarded. This step above was repeated (The buffer breaks away all the impurities because ethanol has been added to the wash buffer- lysis). Each of the test samples were added into a binding column and spun; the nucleic acid binds to the silica membrane, allowing other particles to pass through. After discarding the flow through, the binding column and collection tubes were spun at 12,000 rpm (this was done twice) changing the orientation after the first spin.

The binding columns were transferred into new Eppendorf tubes after checking for clarity (no wets in it) because ethanol wasdegrade RNA if eluted while the collection tubes were discarded. Next, 35 µl of elusion buffer was directly added into the membrane and capped, allowed to sit for five minutes at room temperature and then centrifuged at 10,000rpm for 1 minute and immediately placed on ice block. Finally, the RNA was collected (eluded) in the Eppendoff tubes.

2.8.3 Complementary DNA Synthesis / 1st Round Reverse Transcription - Polymerase Chain Reaction (RT-PCR)

RNA was extracted from a 140 µl mixture (135 µl serum and 5 µl MS2 phage-internal control) by using the QIAmp Viral RNA kit (Qiagen, Hilden, Germany) and the QiaCUBE robotic machine according to the manufacturer's instruction. The extracted products were stored at 80 ◦C before use. During this first step of PCR, the PCR tubes will belabelled and 3µl of PCR graded water (deionised water) was added into each tube and 2µl of primer 4084(HEV-38 5'-GAGGCYATGGTSGAGAARG-3') and primer 4622 (HEV-39 5'-GCCATGTTCCAGACRGTRTTCC -3') was added to each of the tubes. This was to amplify a 240-nucleotide region of the 5′ untranslated conserved region (UTR) of the HCV genome, followed by addition of 15ul RNA in to three test samples labelled PCR tubes and 15ul of graded PCR water was added to the negative control PCR tube (it does not have RNA).

The RT-PCR conditions were set in thermo cycler (PTC – 100TM MJ – Research, INC, Peltier) as follows; reverse transcription was activated for 5 minutes at 94ºC; pre- denaturation for 30 seconds at 94ºC; denaturation for 30 seconds at 53ºC, annealing for 30 seconds at 72ºC and extension for 30 seconds at 94ºC (30 cycles were performed by going back to step two) and final extension for 5 minutes at 72ºC.

2.8.3.1 Polymerase Chain Reaction

Using AccuPowerHotStart PCR PreMix PCR kit by Bioneer Inc. USA., the product of the first amplification reaction of the test samples were used as the template for the second PCR. During this second PCR, four PCR tubes were labelled and 16µl of PCR graded water (DH_2O) were added into each tube and 2µl of the first round PCR product which served as the template, was added followed by addition of 2µl of second primer (HEV-30 5'- CCGACAGAATTGATTTCGTCGG -3') and (HEV-31 5'- GTCTTGGARTACTGCTGR -3') was added to each of the tubes containing the product of first PCR. This is to amplify a 244-nucleotide region of the 5′ untranslated conserved region (UTR) of the HEV genome.

Thermo cycler (PTC – 100TM MJ – Research, INC, Peltier) conditions were set as follows; initial PCR activation steps for 5 minutes at 94ºC, pre-denaturation for 30 seconds at 94ºC; denaturation for 30 seconds at 60ºC, annealing for 30 seconds at 72ºC and extension for 30 seconds at 94ºC (35 cycles was performed by going back to step two) and final extension for 5 minutes at 72ºC.

2.8.3.2 Gel Electrophoresis and Visualization

PCR products of the 3 reactions were confirmed on 2% agarose gel and visualized under ultraviolet light. The gel was prepared by weighing 2.2g of agarose (QD LE Agarose, Green Bio Research, USA) into 100mL of 1X Tris Acetate EDTA (TAE) buffer in a conical flask. The slurry was heated in a microwave for two minutes to properly dissolve the agarose. The solution was then allowed to cool to 60^0C. Ethidium bromide stain - intercalating agent (12µL) was added and mixed thoroughly by rocking. Casting of the molten gel was done by placing a comb on the mould and then gently pouring the molten agarose into the mould.

The gel was allowed to solidify for 30 minutes after which the comb were removed to create wells for the molecular weight ladder and the amplified DNA samples. The tray together with the solidified gel was placed in an electrophoresis tank. A 25bp DNA molecular weight marker of 6µL was loaded into the first wells to enable size estimation of the resolved bands.

About 100 mL TBE buffer was then poured into the tank to cover the cast. Five microlitres of the PCR products (DNA) was then loaded slowly into their respective wells in the submerged gel. electromotive force were applied to the gel and was run under a constant voltage of 120 for 30 minutes. After the 30 minutes, the gel was removed from the gel box, excess buffer from the surface of the gel was drained off and the gel tray placed on paper towels to absorb any extra running buffer. The separated DNA fragments in the gel was visualized by illumination with UV light and pictures were taken with a gel documentation system (Gel Doc 2000, BIORAD, USA) to determine the 244bp of the untranslated conserved region.

2.9 Statistical Analysis

Data entry and statistical analysis was performed with Statistical Packages for the Social Sciences (SPSS) (SPSS, Inc., Chicago, IL). Descriptive data were presented as simple summaries in tables, frequencies and bar charts. Chi-square test was used where appropriate to establish statistically significant differences between participants' variables and prevalence rates. Probability values (p-values) ≤ 0.05 was considered significant.

III. RESULTS AND DISCUSSION

3.1 Results

Table 3.1: Sociodemographic Characteristics of Hepatitis E and C Co-infection among 289 Seropositive HIV Patients Accessing Care at Federal Medical Centre, Keffi, Nigeria

Characteristics	Total number examined (n = 289)	HEV-HIV Seropositivity n = 37	HCV-HIV Seropositivity n = 20	HIV-HEV-HCV Seropositivity n = 27
Age (years):				
18-30	140	20 (14.3%)	6 (4.2%)	7 (5.0%)
31-40	60	10 (16.7%)	4 (6.7%)	5 (8.3%)
41-50	46	5 (10.9%)	7 (15.2%)	8 (17.4%)
>50	43	2 (4.6%)	3 (6.7%)	7 (16.3%)
Gender:				
Male	109	15 (13.8%)	9 (8.2%)	12 (11.0%)
Female	180	22 (12.2%)	11 (6.1%)	15 (8.3%)

Table 3.1 presents the sociodemographic characteristics of the study participants, including age and gender, and their association with HEV and HCV co-infection among HIV seropositive patients. The results show that the prevalence of HEV and HCV co-infection varies across different age groups and gender.

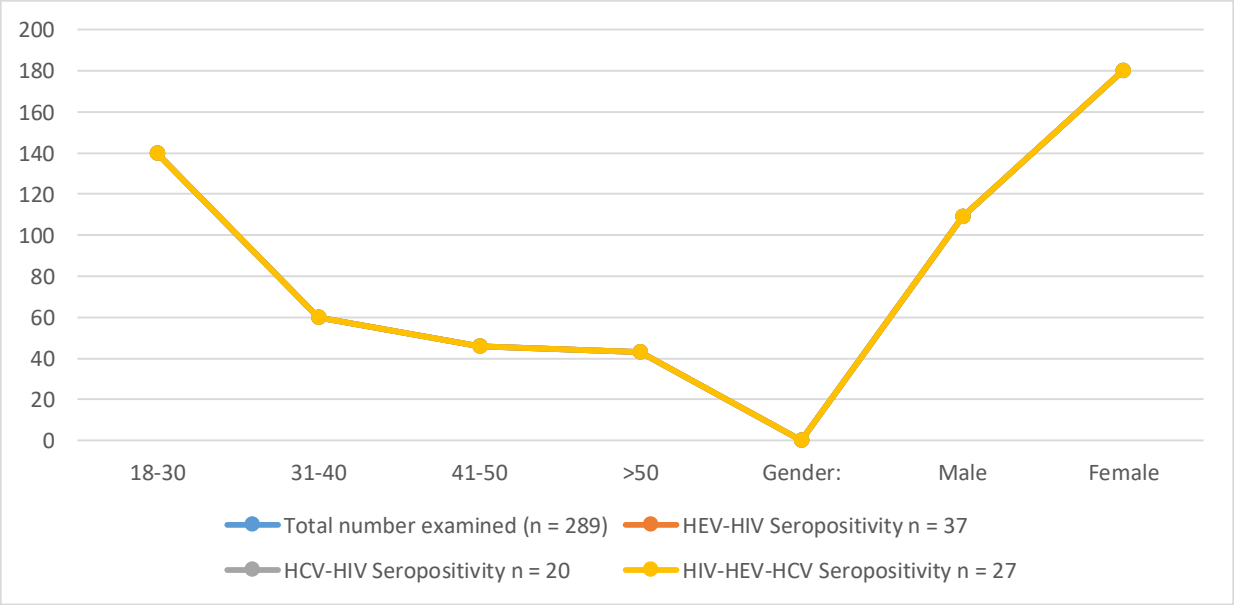

Figure 3.1: Sociodemographic Characteristics of Hepatitis E and C Co-infection among 289 Seropositive HIV Patients Accessing Care at Federal Medical Centre, Keffi, Nigeria

Table 3.2: Baseline Characteristics of HCV and HEV Markers in Relation to Gender among Seropositive 289 HIV Infected Adults Accessing Care at Federal Medical Center, Keffi, North Central Nigeria

Viral Infection Type	Baseline Characteristic	P-value	Female n = 180	Male n = 109
HEV:		**0.002**		
	Positive		22 (12.2%)	15 (13.8%)
	Negative		158 (87.7%)	94 (86.2%)
HevAg:		**0.456**		
	Positive		22 (12.2%)	10 (9.17%)
	Negative		158 (87.7%)	94 (86.2%)
HCV Ab:		**0.568**		
	Positive		11 (6.1%)	9 (8.2%)
	Negative		169 (93.9%)	100 (100%)

Table 3.2 presents the baseline characteristics of HCV and HEV markers in relation to gender among seropositive HIV infected adults. The results show that there is a significant association between HEV infection and gender (p = 0.002).

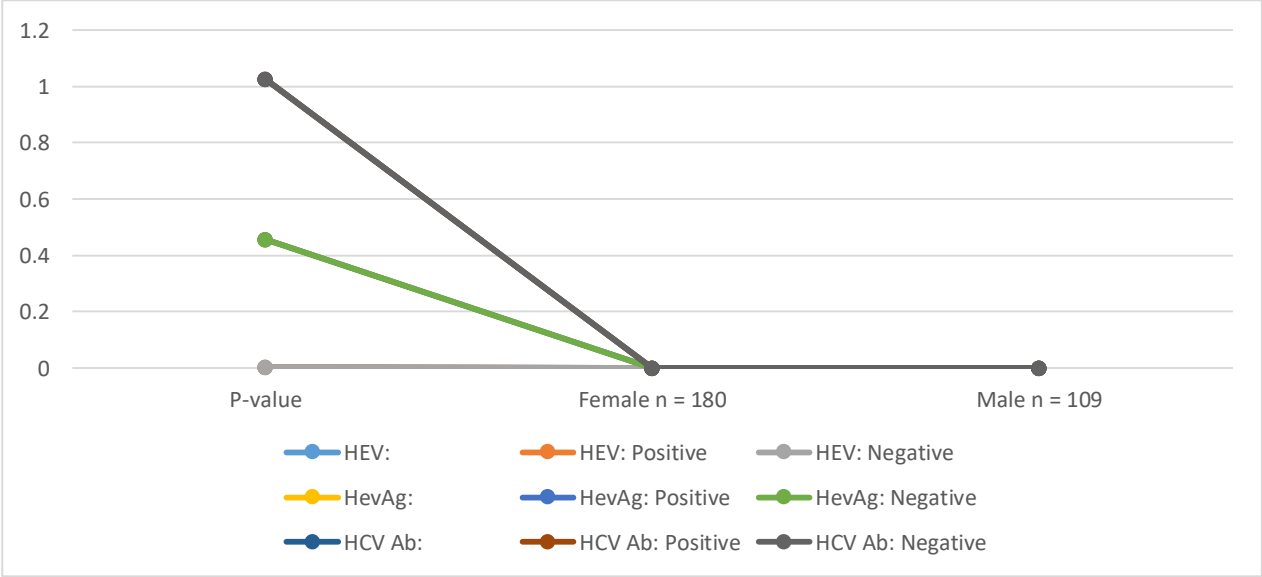

Figure 3.2: Baseline Characteristics of HCV and HEV Markers in Relation to Gender among Seropositive 289 HIV Infected Adults Accessing Care at Federal Medical Center, Keffi, North Central Nigeria

Table 3.3: Associated Risk Factors of HEV, HCV Co-infection among HIV Infected Adults Accessing Care at Federal Medical Center, Keffi, Nasarawa State

Risk Factors	Hepatitis E Surface Antigen n = (%)	Hepatitis C Antibodies n (%)	Hepatitis E/C Co-infection HEV (+) OR (95% CI)	HCV ab (+) OR (95% CI)	HEV/HCV (+) OR (95% CI)
Age (years):					
18-30	20 (14.3%)	1	6 (4.2%)	1	7 (5.0)
31-40	10 (16.7%)	0.79 (0.36-2.0)	4 (6.7%)	1.67 (0.3-9.2)	5 (8.3%)

Table 3.3 presents the associated risk factors of HEV and HCV co-infection among HIV infected adults. The results show that age, gender, and CD4+ T cell count are associated with HEV and HCV co-infection.

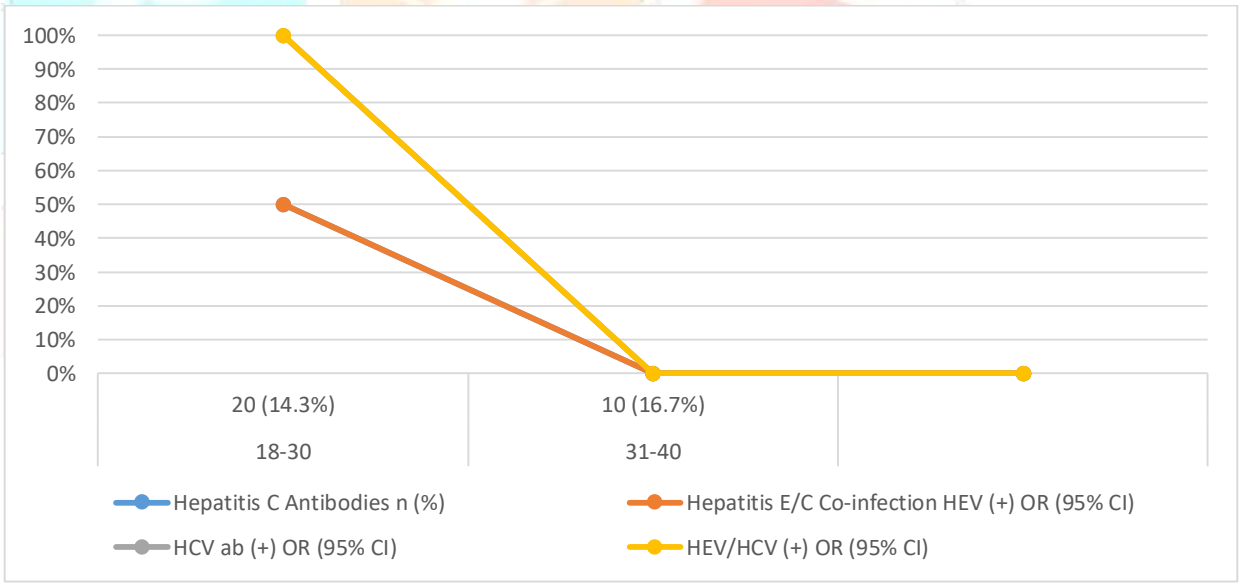

Figure 3.3: Associated Risk Factors of HEV, HCV Co-infection among HIV Infected Adults Accessing Care at Federal Medical Center, Keffi, Nasarawa State

Table 3.4: Immunological and Virological Parameters of HEV-HCV Co-infected with 289 HIV Drug Experience HIV Seropositive Patients Attending FMC Keffi, Nasarawa State, North Central Nigeria

Parameter	HEV Status	HevAg Status	HCV Ab Status	HEV-HCV-Ab Status
Log (HIV Viral Load)	8.98 (7.73-9.8)	0.022†	9.6 (6.84-10.8)	0.031†
CD4+ T lymphocyte count (cells/µl)	239.5 (200.0-390.0)	0.007‡	260.5 (130.0-298.5)	0.018‡

Table 3.4 presents the immunological and virological parameters of HEV-HCV co-infected HIV seropositive patients. The results show that there is a significant association between HEV and HCV co-infection and HIV viral load and CD4+ T cell count.

3.2 Discussion

The present study investigated the prevalence of HEV and HCV co-infection among HIV-seropositive individuals attending Federal Medical Centre, Keffi, Nigeria. The results showed that out of 289 samples tested, 37 (12.8%) were positive for HEV and 20 (6.9%) were positive for HCV antibodies. The prevalence of HEV-HCV co-infection was 9.3% (27/289). These findings are consistent with previous studies that have reported a high prevalence of HEV and HCV co-infection among HIV-infected individuals (Otegbayo *et al.*, 2017; Tadele *et al.*, 2019).

The prevalence of HEV infection in this study (12.8%) is higher than the 7.3% reported in a study conducted in Ghana (Amponsah-Dacosta *et al*., 2018), but lower than the 18.2% reported in a study conducted in Ethiopia (Tadele *et al*., 2019). The prevalence of HCV infection in this study (6.9%) is lower than the 10.3% reported in a study conducted in Nigeria (Otegbayo *et al.*, 2017), but higher than the 3.2% reported in a study conducted in South Africa (Moyo *et al*., 2018).

The results of this study showed that the prevalence of HEV infection was higher among females (12.2%) compared to males (13.8%), although the difference was not statistically significant ($p > 0.05$). This finding is consistent with a study conducted in Ghana that reported a higher prevalence of HEV among females (15.6%) compared to males (10.3%) (Amponsah-Dacosta *et al.*, 2018). The higher prevalence of HEV among females may be due to the fact that females are more likely to be exposed to HEV through contaminated food and water, as they are often responsible for food preparation and handling (WHO, 2017).

The study also found that the prevalence of HCV infection was higher among individuals with a history of blood transfusion (9.5%) compared to those without a history of blood transfusion (2.0%). This finding is consistent with previous studies that have reported a significant association between blood transfusion and

HCV infection (Alter *et al*., 2018). The higher prevalence of HCV among individuals with a history of blood transfusion may be due to the fact that blood transfusion is a known risk factor for HCV transmission (WHO, 2017).

The results of this study also showed that the CD4+ T cell count was significantly lower among individuals co-infected with HEV and HCV compared to those without co-infection ($p < 0.05$). This finding is consistent with previous studies that have reported a significant association between HEV and HCV co-infection and immunosuppression (Kumar et al., 2018). The lower CD4+ T cell count among individuals co-infected with HEV and HCV may be due to the fact that HEV and HCV co-infection can lead to increased immune activation and inflammation, which can result in CD4+ T cell depletion (Kumar *et al*., 2018).

The findings of this study highlight the need for routine screening of HEV and HCV co-infection among HIV-infected individuals, particularly those with a history of blood transfusion. The study also emphasizes the importance of implementing effective prevention and control measures to reduce the transmission of HEV and HCV among HIV-infected individuals.

In conclusion, the present study found a high prevalence of HEV and HCV co-infection among HIV-seropositive individuals attending Federal Medical Centre, Keffi, Nigeria. The study highlights the need for routine screening of HEV and HCV co-infection among HIV-infected individuals and the implementation of effective prevention and control measures to reduce the transmission of HEV and HCV.

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Authors' Contributions

JMM, DI, OMU and IAA conceptualized the study. JMM, ZZ, ID and OMU designed the study. OMU, ID, JMM and ZZ participated in fieldwork and data collection. DI, OMU, JMM and IAA performed the data analysis and interpreted the data. IAA, ZZ, ID, OMU and JMM prepared the first draft of the manuscript. All authors contributed to the development of the final manuscript and approved its submission.

Disclosure of Conflict of Interest

The authors declare that there is no conflict of interest.

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