Pattern of HCV Genotypes in HIV/HCV Co-Infected Patients on Antiretroviral Therapy in Nigeria

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ABSTRACT

At least 33 million people worldwide are living with human immunodeficiency virus (HIV) infection, and about 20-30% of these are also infected with hepatitis C virus (HCV). Co-infection with HIV and HCV is a major public health concern. Co-infected persons develop cirrhosis and end-stage liver disease more quickly than individuals infected with HCV only. The particular HCV strain or genotype is a major factor for HCV prognosis. The pattern of HCV genotypes in a cohort of HIV/HCV co-infected patients was investigated.

One hundred (100) adult patients were recruited from the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, with age ranging from 18 to 65 years (58% male). Upon recruitment, they were placed on appropriate antiretroviral drugs; 300 mg tenofevir (TDF), 200 mg emtricitabine (FTC) plus 600 mg efavirenz (EFV) once daily dosage. HCV genotyping was done using the Linear Array hepatitis C virus genotyping kit (Roche Molecular Systems, Inc. USA).

HCV genotyping revealed prevalence of genotypes 1 (65.6%) and 4 (34.4%), respectively. These are the hard-to-treat genotypes that previously required a long duration of HCV therapy until newer drugs were introduced. The nature of HCV genotypes in HIV/HCV co-infected people has serious implications for further HCV therapy. These findings are pertinent for decisions about the best possible time for and kind of HCV treatment in the setting of co-morbid HIV infection.

Keywords: HCV genotype, HIV-, co-infection, antiretroviral.

I. INTRODUCTION

Hepatitis C virus (HCV) is a major public health concern with significant worldwide morbidity and mortality. This infectious disease represents a major public health problem, with greater than 185 million cases worldwide [1]. The World Health Organization (WHO) estimates that there are between 3 to 4 million new cases per year. HCV is thought to be a “viral time bomb” due to the long-term sequel of infection. Currently, there is no approved vaccine available and approximately 80% of individuals infected by the virus develop chronic disease. It is a risk factor for fibrosis, cirrhosis, liver failure, liver cancer as well as other medical complications (e.g., diabetes). HCV is a small (55-65 nm in size), enveloped, positive-sense single-stranded RNA virus of the family flaviviridae [2].

Until recently, treatment has been based on the use of pegylated interferon (PEG-IFN) alpha-2a or alpha-2b plus ribavirin (RBV). While effective, it results in a cure for only 50% of patients. Moreover, treatment is long (24 – 48 weeks or longer), and it results in severe side effects e.g., depression, anemia. Newer treatments called direct acting antivirals (DAAs) are available with promising results (over 90% cure rates), however majorly for genotype 1. The apparent silent nature of the disease (until the last stages) has led to seeming neglect of the serious impact it can have. Deaths resulting from chronic HCV are mostly due to sequelae in terms of cirrhosis and hepatocellular carcinoma (HCC). In Nigeria, prevalence studies on selected populations have been carried out [3]-[7], with prevalence ranging from 3.4% to 20%. The Federal Ministry of Health however, has stated that prevalence of hepatitis C virus in Nigeria is at 2.2% [8].

HIV infection leads to low levels of CD4+ T cells. When CD4+ T cell numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections [9]-[10]. The introduction of antiretroviral therapy (ART) led to remarkable reduction in number of AIDS-related deaths. While ART results in significantly decreased HIV RNA levels and subsequent increases in CD4 cell count, a paradoxical increase in HCV RNA levels is frequently observed among HCV/HIV co-infected persons initiating ART [11]. Possible explanations for this phenomenon include enhanced lysis of HCV-infected cells by cytotoxic T lymphocytes and release of HCV particles into the plasma.
reduced competition by HIV for HCV entry receptors, reduced HIV-mediated induction of endogenous interferon alpha (IFNα) that would otherwise antagonize HCV replication. Additionally increased cellular injury as a result of antiretroviral-induced hepatotoxicity and/or increased replication of HCV in extra hepatic cells, once HIV levels in those cells have been suppressed.

HIV and HCV co-infection is common and on the rise. Until recently, the clinical course of HCV infection in co-infected individuals was overshadowed by the high morbidity and mortality of HIV. With the introduction of ART and its associated improvements in survival [12], HCV has now emerged as a significant comorbid disease in co-infected patients [13], [14]. HCV has a more progressive course in co-infected patients compared with those with HCV mono-infection. Furthermore, due to shared modes of transmission, HCV is common in HIV patients.

Globally, there are 8 fully classified HCV genotypes presently (1-8) and 67 subtypes [15], but genotype 7 has only been reported in Canada, from a Central African immigrant [16]-[17]. These subtypes (quasispecies) are usually designated with alphabetical suffixes.

The efficacy of current hepatitis C therapy in HIV/HCV co-infected (or mono-infected) patients is dependent on HCV genotype. Therefore, the objective of this study was to determine the prevalent genotypes of HCV in the often-marginalized HIV/HCV co-infected group in Nigeria.

II. MATERIALS AND METHODS

A. Ethical Considerations

Approval for the study was sought and obtained from the Ethics and Research Committee (Institutional Review Board) of Nigerian Institute of Medical Research (NIMR) Yaba Local Government Area (LGA), Lagos State, Nigeria, with reference number IRB-11-0147. Written informed consent was obtained from all participants.

B. Study Design and Setting

This was a cross sectional study among adults positive for both HIV and HCV, enrolled into the ARV treatment programme of NIMR.

The study was conducted at NIMR, Lagos State, Nigeria. NIMR is the apex medical research institution in Nigeria charged with the responsibility to conduct research into diseases of public health importance in the country.

The centre currently provides comprehensive HIV care, treatment and support for over 7,000 individuals. The majority (65%) of them are from Lagos and Ogun States, while the rest are from neighboring states of Oyo, Ekiti, Ondo and Edo and from neighboring West African countries [18]. Unfortunately access to hepatitis B and C management was unavailable until 2014, when a hepatitis clinic led by a gastroenterologist was formed.

C. Specimen Collection

Blood samples were systematically collected from consented, screened patients who had been enrolled, from January 2010 and June 2011, and followed up for 2 years (24 months); ending between December 2011 and June 2013.

Ten milliliters of venous blood were collected from patients into EDTA vacutainers. All samples were centrifuged at 3,500 rpm for 10 minutes within 3 hours of collection. Plasma was separated and stored at -80°C. Drug pick-ups were obtained from the Pharmacy section of the Clinic, while laboratory analyses were carried out at the Centre for Human Virology and Genomics (CHVG). CHVG is a National Reference Laboratory for HIV, and an ISO-15189 certified, accredited (by the South African National Accreditation System (SANAS)) and WHO prequalification evaluating laboratory.

D. Study Participants, Inclusion and Exclusion Criteria

The participants consisted of adult patients who accessed NIMR for HIV care and monitoring. It consisted of 100 (58 male, 42 female) HIV/HCV co-infected adults. Age of the patients ranged from 18 to 65 years. Inclusion criteria were adult patients positive for both HIV and HCV. Exclusion criteria included patients with HIV/HBV, HBV/HCV co-infections, HIV/HBV/HCV tri-infections and HBV, HCV or HIV-2 mono-infections.

E. Baseline Screening Assays

Human Immunodeficiency Virus type-1 (HIV-1): Patients were confirmed HIV-1 positive using 100 microliter (μl) blood plasma and the Enzyme-linked immuno-blotting technique (ELISA) and kits (Immunetics, Boston, USA) following manufacturer’s instructions. Western Blot was initially used until the National Algorithm was updated.

Hepatitis C virus antibody (HCVAb): Patients were screened for HCVAb. These were assayed using 100 μl blood plasma, and the ELISA technique/kits by DIAPRO (DIAPRO Diagnostic Bioprobes, Milano, Italy) following manufacturer’s instructions.

F. Drug Therapy and Dosage

After baseline analysis of patient samples, drug eligible patients were placed on selected antiretroviral therapy (ART) according to national guidelines; Tenofovir (TDF) (300 mg) and Emtricitabine (FTC) (200 mg) (trade name Truvada) plus Efavirenz (EFV) (600 mg) once daily dosage. The drugs were chosen from the nucleoside reverse transcriptase inhibitor (NRTI) and non-nucleoside reverse transcriptase inhibitor (NNRTI) classes of antiretrovirals.

G. HCV Genotyping Assay

Polymerase chain reaction (PCR) reagent preparation: The COBAS Amplicor HCV Amplification kit, version 2.0 (Roche Molecular Systems, Inc., Branchburg, NJ, USA) was used. The manual steps were as follows: Working master mix was prepared by adding 100 μl of HCV manganese ion (Mn2+) each; to 10 vials of HCV master mix (controls inclusive). The tubes were recapped and mixed well by inverting 10 times. A pink dye in the HCV Mn2+ was for visual confirmation that Mn2+ has been added to HCV master mix. Working master mix (50 μl) was added into each labelled Microamp tube using a 200 μl pipettor with aerosol barrier tip, and kept in the fridge until needed.

H. Specimen and Control Preparation

The Amplicor HCV specimen preparation and COBAS Amplicor HCV controls kits, version 2.0 (Roche Molecular
Systems, Inc., Branchburg, NJ, USA) was used for sample extraction. Lysis reagent was prepared by vortexing HCV internal control for 10 seconds, and adding 100 μl of it to one bottle of HCV lysis and mixed well. Lysis reagent (400 μl) was added to each labelled tube and capped. Vortexed normal human plasma (NHP) was added (200 μl) to each of the two control tubes, capped and vortexed for 5 seconds. Twenty μl of HCV negative and positive controls were added to HCV (-) C and HCV (+) C tubes both containing lysis reagent and NHP, capped and vortexed. Next 200 μl of each specimen was added to appropriately labelled tubes containing lysis reagent, capped and vortexed for 5 seconds.

All tubes were incubated in dry heat block for 10 minutes at 60°C and vortexed. Isopropanol (100%, 600 μl) was added to each tube and vortexed. Next, they were incubated for 2 minutes at room temperature, and centrifugated at 12,700 rpm for 15 minutes. Supernatant was removed from each tube and 1 ml of 70% ethanol was added and vortexed. Tubes were placed in a micro-centrifuge for 5 minutes at 12,700 rpm. Following that, the supernatant was carefully removed without disturbing the pellet. HCV diluent (200 μl) was added to each tube and vortexed vigorously. Fifty μl of each processed specimen and control were added to appropriate microamp tubes containing working master mix.

I. PCR of Extracted HCV RNA

Microamp tubes (from the previous section) were placed into the thermocycler and a previously programmed cycling setting, ‘hcv q’ was selected and started. The reaction progressed for 1 hour 30 minutes. At the end of the PCR cycles, it entered a hold temperature of 90°C. The tubes were removed, and 100 μl of denaturation solution was added quickly to all tubes. The denatured amplicons proceeded to Linear Array detection.

J. Linear Array Detection

The Linear Array HCV Genotyping Test kit (Roche Molecular Systems, Inc., Branchburg, NJ, USA) composed of genotyping strips (Nylon strip coated with 9 HCV genotype specific DNA probes) and the Linear Array detection reagents (Roche Molecular Systems, Inc., Branchburg, NJ, USA), and all were used according to manufacturer’s instructions.

III. RESULTS

A total of 245 HCV genotyping assays were conducted in the 24 months study period, among which 100 were from HIV/HCV co-infected patients.

A. Socio-Demographic Characteristics

There was an almost equal distribution of males and females (M: F; 58:42) in the study population. The mean age of the study group was 34 ± 10.4 years. They were majorly (46%) of the youthful age bracket of 25 to 39 years, mostly non-alcohol drinkers and married (89% and 71% respectively). A larger proportion (78%) had an educational level of Secondary and above and employed (86%). The socio-demographic characteristics of the 100 HIV/HCV co-infected participants are shown in Table I.

### TABLE I: SOCIO-DEMOGRAPHIC CHARACTERISTICS OF THE HIV/HCV STUDY POPULATION

| Variables                  | Total (%) | Male (%) | Female (%) | P Value |
|----------------------------|-----------|----------|------------|---------|
| Participants               | 100 (100) | 58 (58)  | 42 (42)    |         |
| Age in Years (mean)        | 34 (±10.4)| 33 (±9.2)| 34 (±9.7)  | 0.625   |
| 18-24                      | 31 (31)   | 19 (19)  | 12 (12)    |         |
| 25-39                      | 46 (46)   | 28 (28)  | 18 (18)    | 0.371   |
| 40 and above               | 23 (23)   | 11 (11)  | 12 (12)    |         |
| Educational Status         |           |          |            |         |
| < Secondary Education      | 22 (22)   | 9 (9)    | 13 (13)    | 0.010*  |
| Secondary Education >      | 78 (78)   | 41 (41)  | 37 (37)    |         |
| Work Status                |           |          |            |         |
| Employed                   | 86 (86)   | 51 (51)  | 35 (35)    | 0.046*  |
| Unemployed                 | 14 (14)   | 8 (8)    | 6 (6)      |         |

*Statistically significant.

A. HIV Serology

HIV test was conducted according to Nigerian National HIV testing and counselling guidelines in persons with unconfirmed HIV status before enrolment into the study. Diagnosis was based on positive test on double ELISA based algorithm. However, before initiation of antiretroviral therapy, the initial HIV test was confirmed by Western Blot. A cross-section of the results is shown in Fig. 1.

![Fig. 1. Cross-section of HIV immunoblotting test strips of samples from 100 HIV/HCV patients. Definitive HIV-1 glycoproteins (GP 160, 110 and 41), proteases (P 68/66, 55, 40 24/25), internal control (IC) bands, Negative control (NC) and Positive control (PC) are shown.](image)

B. HCV Genotypes

HCV genotypes of the HIV/HCV co-infected patients were determined. A cross section of the HCV genotype test strips containing the different genotypes are shown in Fig. 2. Genotypes 1 and 4 are clearly indicated along with the positive and negative controls. The frequencies of the genotypes are depicted in the bar chart in Fig. 3.

![Fig. 2. Cross section of test result strips of two HCV genotypes. The lowest blue bar (internal control) is common to all genotypes, while genotype 1 and the positive control have similar indicative bars, but fainter for the latter.](image)
Fig. 3. Bar chart of HCV genotype prevalence of the HIV/HCV co-infected.

IV. DISCUSSION

This study demonstrated that HCV genotypes 1 and 4 were the only genotypes found among HIV/HCV co-infection, with a dominance of genotype 1. They are among three hard-to-treat genotypes (including genotype 1, 4, and 6); by implication they required longer duration of HCV antiviral therapy to attain sustained viral response (SVR), before the introduction of pan-genotypic DAAs. A similar Spanish study of HIV-HCV co-infected persons gave a prevalence of 78% for genotype 1 [18]. This study’s report differs from a previous HCV genotype Nigerian study carried out on HCV mono-infected patients [19]. That study found genotypes 1, 2, 3, 4 and 6 and also dual genotypes. However, it correlates with the present study in the fact of similar prevalence of genotype 1 which was 64.7% in that study [19]. There is therefore a heterogeneity of HCV genotypes in the population, but with a preponderance of genotype 1.

Other parts of the world have reported the same heterogeneous nature of HCV genotypes. Studies in Brazil, Italy, Spain and the United States have reported several genotypes even in HCV mono-infection [20]. If this trend is proven, the recognition of a steady accumulation of difficult-to-treat patients may stress the need for prioritizing new anti-HCV drugs in the co-infected population, in whose progression to end-stage liver disease occurs faster and a substantial proportion of them already have cirrhosis. The genotypes in HCV disease have invariably complicated its treatment, and the divergence of HCV genotypes may explain features of their distribution. In the new era of pan-genotypic drugs (active against most of the existing HCV genotypes) for HCV, there may be said to be little necessity to investigate genotypes of HCV, but it is nonetheless pertinent if it can be accomplished for clinical management, epidemiological surveillance and research purposes.

In conclusion, co-infection with HIV and HCV is a unique challenge. Appropriate country-specific prevention, diagnosis and treatment strategies to reduce the disease burden of HCV and in HIV co-infection needs to adopted. HCV disease put pressure on economies of low-income countries like Nigeria. The genotypes are pertinent in decisions about HCV treatment in the setting of co-morbid HIV infection.

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CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

REFERENCES

[1] Mohd-Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. Hepatology. 2013; 57(4): 1333-1342.
[2] Simmonds P. Viral heterogeneity of the hepatitis C virus. Journal of Hepatology. 1999; 31: 54-60.
[3] Lesi OA, Kehinde MO. Hepatitis C virus Infection in patients with sickle cell anaemia at the Lagos University Hospital. Nigerian Postgraduate Medical Journal. 2003; 10: 79-83.
[4] Forbi JC, Gabadi S, Alabi R, Ipeorepolu HO, Pam CR, Entona PE, et al. The role of triple infection with hepatitis B virus, hepatitis C virus and Human Immunodeficiency Virus-Type 1 on CD4+ lymphocyte levels in the highly HIV infected population of North-Central Nigeria. Memórias do Instituto Oswaldo Cruz. 2007; 102: 535-537.
[5] Ejiofor OS, Ike BC, Emoji II, Ifekuma AN, Buekukwu GC, Emechebe G. The role of blood transfusion on the prevalence of hepatitis C virus antibodies in children with sickle cell anaemia in Enugu, South East Nigeria. Nigerian Journal of Clinical Practice. 2009; 12: 355-358.
[6] Ejiofor OS, Emechebe GO, Igwe WC, Ikadikie CO, Ubajaka CF. Hepatitis C virus infection in Nigerians. Nigerian Medical Journal. 2010; 51: 173-6.
[7] Achinge GI, Mala AO, Bhaave PT, Bitto TT, Shaahu VN, Mohammed H, et al. Prevalence of Hepatitis C in Makurdi, North Central Nigeria. IOSR Journal of Dental and Medical Sciences. 2013; 7 (5): 6 – 10.
[8] Guidelines for the Prevention, Treatment and Care of Viral Hepatitis in Nigeria. Federal Ministry of Health-National AIDS/STIs Control Programme. 2016.
[9] Ebuheh OAT, Balogun MO, Audu RA, Idigbe EO. Osmotic fragility and Na+ - K+ ATPase activity of erythrocytes of HIV/AIDS patients. Saudi Medical Journal. 2003; 24 (12): 1412-1414.
[10] Cunningham A, Donaghy H, Harman A, Kim M, Turville S. Manipulation of dendritic cell function by viruses. Current opinion in Microbiology. 2010; 13(4): 524-529.
[11] Cooper C, Cameron D. Review of the effect of highly active antiretroviral therapy on hepatitis C virus (HCV) RNA levels in human immunodeficiency virus and HCV co infection. Clinical Infectious Disease. 2002; 35: 873-879.
[12] Palella FJ Jr, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection: HIV Outpatient Study Investigators. The New England Journal of Medicine. 1998; 338: 853-860.
[13] Bica I, McGovern B, Dhar R, Stone D, McGowan K, Scheib R, et al. Increasing mortality due to end-stage liver disease in patients with human immunodeficiency virus infection. Clinical Infectious Diseases. 2001; 32: 492-497.
[14] Monga HK, Rodriguez-Barradas MC, Breaux K, Khattak K, Troli CL, Velez M. Hepatitis C virus infection-related morbidity and mortality among patients with human immunodeficiency virus infection. Clinical Infectious Diseases. 2001; 33: 240-247.
[15] Smith DB, Bahl J, Kuiken C, Muershoff AS, Rice CM, Stapleton JT, et al. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. Hepatology. 2014; 59(1): 318–327.
[16] Murphy DG, Williams B, Deschenes M, Hilzennrat M, Nourseau R, Sabbath S. Use of sequence analysis of the NS5B region for routine genotyping of hepatitis C virus with reference to C/E1 and 5′ untranslated region sequences. Journal of Clinical Microbiology. 2007; 45(4): 1102-1112.
[17] Adu RA, Ezechii OC, Onwujeke DJ, Odumutke NN, David AN, Kalejaiye OO, et al. The changing pattern of HIV related deaths in South Western Nigeria. 5th National Conference on HIV/AIDS. Abuja, Nigeria. 2010.
[18] Barreiro P, Labarga P, Fernandez-Montero JV, Poveda E, Mendeza C, Sanchez C, et al. Longitudinal changes in viral RNA concentration in patients with chronic hepatitis C and/or HIV infection in the absence of antiviral therapy. *Journal of Clinical Virology*. 2013; 58: 391-395.

[19] Okwuraiwe AP, Sahu OB, Anomneze E, Audu RA, Ujah IAO. Hepatitis C virus genotypes and viral ribonucleic acid titres in Nigeria. *Nigerian Journal of Gastroenterology and Hepatology*. 2012; 4 (2): 75 - 85.

[20] Petruzzello A, Marigliano S, Loquercio G, Cacciapuoti C. Hepatitis C virus (HCV) genotypes distribution: an epidemiological up-date in Europe. *Infectious Agents and Cancer*. 2016; 11: 53.