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 tenofovir (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.
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]. 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 DIA PRO (DIA PRO 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
Infected participants are shown in Table 1. Socio-demographic characteristics of the 100 HIV/HCV co-infected patients are depicted in the bar chart in Fig. 1. The demographic data is shown in the table below.

Table 1: Socio-Demographic Characteristics of the HIV/HCV Study Population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (%)</th>
<th>Male (%)</th>
<th>Female (%)</th>
<th>P Value</th>
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</thead>
<tbody>
<tr>
<td>Participants</td>
<td>100 (100)</td>
<td>58 (58)</td>
<td>42 (42)</td>
<td></td>
</tr>
<tr>
<td>Age in Years (mean)</td>
<td>34 (±10.4)</td>
<td>33 (±9.2)</td>
<td>34 (±9.7)</td>
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<tr>
<td>18-24</td>
<td>31 (31)</td>
<td>19 (19)</td>
<td>12 (12)</td>
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<tr>
<td>25-39</td>
<td>46 (46)</td>
<td>28 (28)</td>
<td>18 (18)</td>
<td>0.371</td>
</tr>
<tr>
<td>40 and above</td>
<td>23 (23)</td>
<td>11 (11)</td>
<td>12 (12)</td>
<td></td>
</tr>
<tr>
<td>Alcohol intake</td>
<td>Yes</td>
<td>11 (11)</td>
<td>9 (9)</td>
<td>2 (2)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>89 (89)</td>
<td>49 (49)</td>
<td>40 (40)</td>
</tr>
<tr>
<td>Marital Status</td>
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<tr>
<td>Unmarried</td>
<td>29 (29)</td>
<td>18 (18)</td>
<td>11 (11)</td>
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<tr>
<td>Married</td>
<td>71 (71)</td>
<td>36 (36)</td>
<td>35 (35)</td>
<td>0.814*</td>
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<td>9 (9)</td>
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<td>41 (41)</td>
<td>37 (37)</td>
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<td>41 (41)</td>
<td>37 (37)</td>
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<td>Work Status</td>
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<td>86 (86)</td>
<td>51 (51)</td>
<td>35 (35)</td>
<td>0.046*</td>
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<td>Unemployed</td>
<td>14 (14)</td>
<td>8 (8)</td>
<td>6 (6)</td>
<td></td>
</tr>
</tbody>
</table>

*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.

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.
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 virological response (SVR), before the introduction of pan-genotypic DAAAs. 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 whom 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

