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Original article

COMPARISON OF HCV DIAGNOSIS BY ANTIBODY DETECTION AND RNA IN HIV-1 CO INFECTED PATIENTS COMMENCING HAART

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ABSTRACT

Background: Hepatitis C virus (HCV) diagnosis either in isolation or in HIV co-infection has often been laden with misdiagnosis, false positives and negatives being reported frequently. The use of strip tests has been replaced with the more sensitive fourth generatiom enzyme linked immunosorbent assay (EIA) HCV antibody test. However, a definitive HCV diagnosis is best achieved by determination of presence of HCV viral RNA. Objective: The study is to compare two HCV serological and molecular diagnosis techniques, in a cohort of HIV-1/HCV co-infected patients commencing antiretroviral therapy. Methods: Samples which tested positive for hepatitis C antibody (HCVAb) using the ELISA technique from 100 HIV-1/HCV patients (58 male, 42 female), aged between 18 and 65 years, with (Dia.Pro Diagnostic BioProbes s.r.l) where retrospectively retrieved. PCR based HCV RNA qualitative (COBAS Amplicor, Roche Diagnostics, Branchburg, USA) and HCV RNA quantitative (COBAS Ampliprep/COBAS TaqMan, Roche Diagnostics, Branchburg, USA) assays were simultaneously carried out on the aliquots of these samples. Results: Out of the 100 HIV/HCV positive patients, only 64 (64%) were positive on the qualitative and 64 (64%) also with viral titer on quantitative assays (limit of detection 15 IU/ml). The remaining 36 (36%) had negative HCV RNA and 'Not Detected'for the qualitative and quantitative assays respectively. These results tend to indicate an absence of active HCV infection for a significant portion of the HIV-HCV co-infected population. Conclusion: HCVAb testing should not be the only assay used to determine HCV dignosis in Nigeria. Although expensive, requiring instrumentation and longer time factor, HCV RNA (both qualitative and quantitative) is highly recommended, especially for immuno-compromised persons.

1. Introduction

On a global scale, 170 to 200 million people are estimated to be infected with HCV[1]. It has been reported that 30% of human immunodeficiency virus (HIV) people globally are infected with HCV. Most people with HCV feel well and have no obvious observations on physical examination. Therefore detection of HCV plasces a large emphasis on laboratory tests results to diagnose and evaluate therapy[2].

HCV a causative organism for clinical hepatitis and carcinomas, has been poorly or erroneously diagnosed for a long time, due to the RNA nature of the virus. It has very high mutability and constantly changes morphology evading the immune system3. HCV diagnosis by second generation Enzyme linked immunosorbent assay (ELISA) can result in 'false positives' occiuring at a rate of 10 – 20% and are usually seen in the presence of autoimmune disease and low-risk blood donors[3]. On the other hand, 'false negative' results by ELISA may occur in immunosuppressed patients, including those livivng with HIV. In early infection, HCVAb testing may be negative , as antibodies may not develop until 4 – 6 weeks after exposure[3].

Unfortunately, a positive hepatitis C antibody test does not distinguish acute from chronic disease or active from past infection. Therefore, a positive ELISA HCVAb test is a marker that hepatitis C may be present and it should be followed up by confirmatory PCR based HCV RNA (qualitative and/or quantitative)4,5,6. HCV RNA can be detected under 2 weeks of exposure, unlike HCVAb which is detected between 6 to 10 weeks after exposure. Furthermore, in HIV/HCV co-infected patients, the World Health Organisation (WHO) Algorithm states that antiretroviral therapy (ARV) should be started after HIV diagnosis, irrespective of CD4 count, but after HCV RNA conclusive diagnosis has been made7. Unfortunately, this policy directive is not being practised by Nigerian National HIV treatment programmes.

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The objective of this study therefore, was to compare two HCV diagnostic assays or techniques, the serological (antibody) and molecular (RNA genetic material) in a cohort of HIV/HCV co-infected patients commencing ARV therapy.

METHODS

Study Setting

The study was conducted at the Human Virology Laboratory of Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria. The Laboratory is a reference laboratory for HIV in Nigeria, and NIMR is one of the largest HIV/AIDS treatment centers in Nigeria.

Study Design, Ethical Approval and Informed Consent

The study was a retrospective cross sectional study. Ethical approval was obtained from the institutional Review Board (IRB) of NIMR. Informed consent was obtained from all patients at enrolment.

Study population

The study population was derived from randomly selected HIV-1 patients co-infected with HCV as determined by Enzyme Linked Immunosorbent Assay (ELISA or EIA). One hundred HIV-1/HCV co-infected patients (male to female ratio 1:1) being enrolled for HIV treatment, recruited from January to December 2010, were employed for the study.

Inclusion and Exclusion Criteria

Adults patients 18 years and above, that were HIV-1 and HCV positive by serology were used for this study. Exclusion criteria included those that were HIV-1 mono-infected, HIV-1/HBV co-infected and HIV-1/HBV/HCV triple infected.

Laboratory Assays

HIV-1 Serology

Five mls of whole blood obtained by venous puncture and collected in a 5 ml EDTA vacutainer. It was centrifuged at 8000 rcf for 10 minutes to obtain about 2 ml plasma. Positive results were obtained by use of double EIA by Immunetics (Boston, USA) following manufacturer's instructions.

HCV Serology

HCV antibody assay was carried out using a fourth generation EIA test method by Dia.Pro Diagnostic BioProbes s.r.l., Italy, following the manufacturer's instructions.

HCV Molecular Assays

HCV RNA Qualitative Test

From plasma aliquotes obtained previously, 200µl of which was used to assay HCV RNA qualitative test using the COBAS Amplicor analyzer and reagents/kits (Roche Diagnostics, Branchburg, USA). It is a semi-automated assay, composed of a manual extraction of HCV RNA, followed by automated PCR and amplicon detection on the Amplicor analyzer.

HCV RNA Quantitative Test

From plasma aliquotes obtained previously, 1000µl of which was employed to assay HCV RNA quantitative test (viral load) using the COBAS Ampliprep/COBAS Taqman analyzer and reagents/kits (Roche Diagnostics, Branchburg, USA). It is a fully automated assay, composed of automated extraction of HCV RNA (Ampliprep), followed by automated PCR and amplicon detection on the Taqman (real time PCR) analyzer.

RESULTS

The age of the study cohort ranged from 18 to 65 years, with a mean of 34 ± 3.7 years. There were 58 males and 42 females, with a M:F ratio of approximately 1:1. Out of the 100 HIV-HCV co-infected patients, 64 (64%) were HCV RNA positive on qualitative HCV RNA test. The same number (64%) were HCV detectable on the HCV RNA quantitative test, that is had a viral titre. The median HCV RNA for these group was 61,684 IU/ml.

DISCUSSION

Although the optimal strategy for diagnosing chronic HCV infection can vary, all testing algorithms begin with the use of a serologic assay that detects antibodies to HCV. The fourth generation HCV EIA is currently widely used as the screening test to diagnose chronic HCV infection and it has an estimated 98% sensitivity for detecting antiobody to HCV8. In this comparative study, of all the patients that reported HCV antibody positive at enrolment, a relatively large proportion were negative and not detected on molecular assays. A probable reason for the initial seropositivity is that the antibody test detects both current and past infections. Moreover, among HCV positive persons, those who achieve spontaneous viral clearance usually remain HCV Ab positive for a considerable period of time. Confirmatory testing for presence of HCV RNA is therefore needed to distinguish resolved from active, current infections.

Studies by Firdus et al9 and Araujo et al10 reported that HCV immunoassay could not be relied upon as an accurate diagnostic tool for screening HCV infection, especially in high risk group patients such as HIV co-infected, haemodialysis and thalassaemic patients.

A positive HCV report in HIV infected individuals requires the individual to have a consultation with a hepatologist or gastroenterologist. Co-infected patients are usually managed intricately, with lesser hepatotoxic drugs and early drug regimen commencement irrespective of the CD4 count. With adequate HIV treatment (reaching not detected levels), it could be interrupted to undergo the 24 or 48 weeks of HCV treatment depending on the HCV genotype. It has been debated that HIV-1/HCV co-infection has different outcomes than its mono-infected counterparts.

Among those with HCV viral load detected, viral titre was fairly high on average (61,684 IU/ml) and will require antiviral therapy. Traditional HCV therapy, peg-interferon plus ribavirin, has a notoriously brutal toxicity profile and a depressingly low success rate in HIV co-infected people. Newer drugs in a class called “direct acting antivirals” are coming into limelight (e.g. Ledipsavir and Sofosbuvir) having better prognosis and cure rates, but expensive. However, for the co-infected patients, which treatment to initiate first, pill burden and drug interactions become contending issues.

HCV diagnosis is an evolving laboratory technique continuously undergoing improvement. There has been better diagnostic capacity of the second, third and at present, fourth generation screening compared to the first generation tests. To confirm and rule out current HCV infection, it is recommended that persons with undetectable HCV RNA have a follow-up HCV RNA quantitative test done six months later.

CONCLUSION

The identification of HCV RNA status (qualitative and/or quantitative) is more advantageous than serology although expensive. Overall HCV RNA is a more accurate diagnosis and important for further clinical evaluations and is recommended.

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