Research Article

Human Immunodeficiency Virus and Hepatitis C Virus Co-infection in Cameroon: Investigation of the Genetic Diversity and Virulent Circulating Strains

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ABSTRACT

Background: RNA virus infections represent a significant cause of illness and death in vertebrates. Specifically in humans, RNA viruses are responsible for a wide range of acute, chronic, emerging and re-emerging infections. HIV and HCV rank as some of the most severe RNA viruse infections facing Africa. **Methods**: To determine genotypes and subtypes of HIV and HCV among co-infected patients in Cameroon, viral RNA was isolated from HIV/HCV co-infected individuals, in Douala, Cameroon. A total of 36 HIV/HCV co-infected isolates (22 from volunteer blood donors and 14 from people living with HIV/AIDS not yet on antiretroviral treatment) were analyzed using molecular biology techniques that involved RT-PCR, gene/TOPO cloning, DNA sequencing, and bioinformatics tools for sequence management and analysis. Epidemiological data were examined as well. **Results**: Results show that HIV strains isolated belong to the circulating recombinant forms CRF02_AG, whereas HCV isolates from Cameroon belong to genotypes 1, 2, and 4. The corresponding HCV subtypes investigated were 1a, 1b, 1c, 2a, 2c, 2k, and 4a. Subtypes 1a and 1b, most frequently found in developed countries, also circulate in Cameroon. Epidemiologic data show that HIV/HCV co-infected patients are older than HIV mono-infected patients. **Conclusions**: These results indicate that HIV/HCV co-infection represent a significant threat in Cameroon. There is evidence of genetic diversity of HIV and HCV; virulent hepatitis C virus subtypes *1a* and *1b* circulate in Cameroon. An epidemiological and molecular database on HIV and HCV is necessary for the development of further intervention in Cameroon as an imperative for monitoring disease progression.

Key words: HIV; HCV; Co-infection ; Genotypes ; Virulent.

RÉSUMÉ

Contexte: L'infection par les virus à ARN représente une cause importante de maladie et de décès chez les vertébrés. En particulier chez les humains, les virus à ARN sont responsables d'une large gamme de maladies infectieuses aiguës, chroniques, émergentes et ré-émergentes. Le VIH et le VHC sont parmi ces virus à ARN qui plus posent de graves problèmes à l'Afrique. **Méthodes**: Pour déterminer les génotypes et sous-types de VIH et de VHC chez les patients co-infectés au Cameroun, l'ARN viral a été isolé à partir des spécimens de VIH et VHC chez les personnes co-infectées à Douala au Cameroun. Un total de 36 isolats co-infectés VIH/VHC (22 chez les donneurs bénévoles de sang et 14 chez des personnes vivant avec le VIH/SIDA, naïfs de traitement) ont été analysés en utilisant des techniques de biologie moléculaire, RT-PCR, TOPO clonage de gène, séquençage de l'ADN et outils bio-informatiques pour la gestion et l'analyse des séquences. Les données épidémiologiques également ont été explorées. **Résultats**: Les résultats montrent que les souches de VIH isolées appartiennent à la forme recombinante circulante CRF02_AG, alors que isolats de HCV au Cameroun sont des génotypes 1, 2 et 4. Les sous-types du VHC correspondant étaient 1a, 1b, 1c, 2a, 2c, 2k, et 4a. Les sous-types 1a et 1b, le plus souvent retrouvé dans les pays développés, circulent aussi au Cameroun. Les données épidémiologiques montrent que les personnes infectées par le VHC et donc co-infectés VIH/VHC, sont plus âgées que les patients mono-infectés VIH. **Conclusions**: Ces résultats indiquent que la co-infection par le VIH et VHC représente une menace importante au Cameroun. Il y-a ainsi des preuves de diversité génétique du VIH et du VHC; les sous-types virulents du VHC, 1a et 1b circulent au Cameroun. Une base de données épidémiologique et moléculaire sur le VIH et le VHC est nécessaire pour plus d'intervention au Cameroun. Plus important encore, il y a une exigence impérative pour la surveillance de la progression de la maladie.

Mots clés: VIH, VHC, Co-infection, Génotypes, Virulent

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INTRODUCTION

HIV/AIDS ranks as one of the most prevalent infectious disease facing mankind in the 21st century. Although global solidarity in the AIDS response during the past decades has generated health gains, HIV/AIDS remains one of the world's most serious health challenges. Sub-Saharan Africa remains most critically affected with nearly 1 in every 20 adults living with HIV (UNAIDS/WHO, 2012). This represents 66% of people living with HIV worldwide, in a population with just over 10% of the world population. HIV is primarily characterized by a high genetic variability due to the low fidelity of the viral reverse transcriptase (RT) that lacks proofreading activity, and to the high virus turnover *in vivo* of 10^9 virions per day (Mansky and Temin, 1995). Because reverse transcriptase switches templates during reverse transcription, virions can give rise to recombinant progeny (Hu and Temin, 1990). Four phylogenetic groups of HIV-1 based on nucleotide sequence analyses have been identified and described: M (major), O (outlier), N (non M, non O), and the most recent discovered group P (1994; Robertson et al., 1995; Simon et al., 1998; Plantier et al., 2009b). Group M viruses are globally prevalent, group O is mostly found in West and Central Africa, groups N and P circulate in Cameroon (Brennan et al., 2008; Plantier et al., 2009b; Vallari et al., 2010; Vallari et al., 2011). Although these new emergent groups have been reported in Africa, HIV-1 group M remains prevalent throughout the world and therefore in Africa. The most recent nomenclature of HIV-1 divides group M viruses into subtypes and sub-subtypes A (A1, A2) – D, F (F1, F2) – H, and J-K. Moreover, 49 groups of Circulating Recombinant Forms (CRFs) of HIV-1 among other 37 first generation recombinants and twelve (12) second generation recombinants circulate throughout the world (Los Alamos Sequence Database, 2008-2011). Most of the CRFs described contain a portion of subtype A: e.g. CRF01_AE; CRF02_AG. Previous studies on HIV genome characterization have reported a high genetic variability of subtypes and different recombinant forms of HIV-1 from the southern, central and northern parts of Cameroon (Ndumbe et al., 1992; Tebit *et al.*, 2002; Nyambi et al., 2002; Ndembi *et al.*, 2004; Yamaguchi *et al.*, 2004; Djuidje Ngounoue, 2009). It is evident that such variations could significantly impact the susceptibility and resistance to antiretroviral drug as well as the performance of diagnostic assay.

In Africa, HIV infection has overshadowed other chronic infections such as Hepatitis C Virus (HCV) infection. However, a critical review of the literature shows that HCV infection is as prevalent as TB, malaria, and Sexually Transmitted Infections (STIs). It is reported that acute HCV infection is followed in 80% of cases by virus persistence, leading to chronic hepatitis that can progress to cirrhosis and hepatocellular carcinoma over several decades. HCV genome is heterogeneous due to the poor fidelity and the lack of proofreading activity of the non-structural NS5B-encoded RNA-dependent RNA polymerase. Isolates from all over the world have now been grouped into main genotypes, each containing several subtypes, based on sequence data (Simmonds *et al*, 2005). Therefore, it is important to study the genetic diversity of HIV and HCV and the correlation between such viral co-infections and co-morbidities.

This study aimed at determining the genotypes and subtypes of HCV and HIV using HIV/HCV co-infected isolates from Cameroonian patients (ages 21-64 from both male and female) using genomics and bioinformatics approaches, as well as exploring epidemiological data, age and prevalence.

MATERIALS AND METHODS

Sample collection, population and research setting: The present study was conducted on

blood samples collected between January 2005 and February 2006 during an epidemiological survey project on HIV and hepatitis sero-prevalence in Douala (Djuidje Ngounoue et al., 2008; Djuidje Ngounoue et al., 2009). A total of 36 HIV/HCV co-infected isolates (22 from volunteer blood donors, and 14 from people living with HIV/AIDS, not yet on antiviral treatment) were collected and subsequently analyzed using molecular biology techniques and bioinformatics tools. Viral RNA were prepared in Cameroon (March-April 2006) and stored at -20°C until transferred to US (July 2008) under safety conditions, on a Material Transfer and Data Sharing Agreement between the institutions. RT-PCR, TOPO cloning, DNA sequencing and bioinformatics techniques were performed at the J. Craig Venter Institute, Maryland, United States.

Ethical Considerations: For the proper conduct of the present study involving humans, the Declaration of Helsinki and the CIOMS International Ethical Review of Epidemiological Studies were fulfilled; Ethical clearance from the Cameroon National Ethics Committee was obtained prior to the implementation of the study; Ethical approval from the Institutional Review Board (IRB) of the J. Craig Venter Institute in the United States was obtained prior to the molecular analysis; Appropriate informed consent was obtained from 90% of participants at the moment of sample collection. The remaining 10% of participants were patients in emergency situation and in unconscious state. Therefore, both head of the hospital and head of the "day care" centre, together with the physicians and nurses in the service agreed with the investigators for their data to be collected.

Primer Designing: Emphasis was on genes used in previous studies for diagnostic, genetic variability and drug resistance. Therefore, primers targeting ***HIV Pol-RT***, ***HCV E2,*** and ***HCV NS5B*** regions on viral genomes (Ndjomou *et al.*, 2003; Njouom *et al.*, 2003; Ndembi *et al.*, 2004; Njouom *et al.*, 2005) were designed and used in the Polymerase Chain Reactions (PCR) techniques.

Gene Amplification, Agarose Gel Electrophoresis and Gel Extraction: *HIV Pol-RT* gene was amplified using RT-PCR coupled with nested PCR. *HCV NS5B* gene was amplified throughout RT-PCR coupled with semi-nested PCR (Applied Biosystems). For *E2 gene,* cDNA was synthesized and amplified. For success and quality control, PCR products were resolved in 1.5% Agarose gel electrophoresis in 1X TAE buffer, with ethidium bromide staining. Positive samples were excised and purified with QIAquick Gel Extraction QIAGEN Kit according to the manufacturer instructions.

Gene Cloning: Purified PCR products were cloned into a TOPO pCR 2.1 vector. To generate recombinant plasmid, the TOPO-cloning reaction was used to transform one-shot TOPO TOP 10 chemically competent *E. coli* that were grown overnight in an appropriate YET 2 medium. Plasmid DNA was prepared from the culture and used for sequencing reactions.

DNA sequencing: Sequencing reactions were done with Big Dye Terminator according to Sanger technique, Big Dye® Terminator Cycle Sequencing Ready Reaction version 3.1 instructions (Applied Biosystems). DNA Sequencing was performed in an Applied Biosystems ABI 3100 DNA Analyzer. Sequencing coverage was up to 297bp for *HIV Pol-RT* gene, 310bp for HCV E2, and 400bp with the HCV NS5B gene.

Sequence Data Management and Analysis: Nucleotide sequences were assembled into consensus sequences using SeqMan Expert Analysis Software implemented in DNASTAR;

Consensus sequences were used for homology searches at the NCBI database using the Nucleotide Basic Local Alignment Search Tool *BLASTN 2.2.18+* program. Nucleotide sequences were aligned using Clustal W implemented in BioEdit sequence alignment. Using consensus sequences, genotypes and subtypes were determined through the genotype reference set at the NCBI database.

RESULTS AND DISCUSSION

Molecular results

The genotyping and sub-typing of HIV *POL-RT* sequences showed that strains isolated are circulating recombinant forms, CRF02_AG. Previous studies documented the circulation of HIV-1 CRF02_AG in Central Africa and in Cameroon using DNA extracted from peripheral mononuclear blood cells (PBMC) as template for HIV genome amplification (Mboudjeka *et al.*, 1999; Lythgo *et al.*, 2000; Tebit *et al.*, 2002; Ndembi *et al.*, 2004; Yamaguchi et al., 2004; Ndembi et al., 2004; Ndembi et al., 2008; Makamche, 2008;). In addition, current findings indicate that CRF02_AG continues to be the predominant strain in Cameroon (Ragupathy et al., 2011; Agyingi et al., 2014). The analysis of HCV *E2* gene displayed the genotype 2, and the HCV *NS5B* gene showed that strains from Cameroon belong to genotypes 1 and 4. Therefore according to the present study, HCV genotypes that circulate in Cameroon are: genotype 1(45%), genotype 2(22%), and genotype 4(33%). The corresponding subtypes investigated were 1a, 1b, 1c, 2a, 2c, 2k, and 4a. Previous studies in Cameroon used the *NS5B* gene for genotyping as well as for genetic diversity studies (Ndjomou *et al.*, 2002; Ndjomou *et al.*, 2003; Njouom *et al.*, 2003; Njouom *et al.*, 2005). Since amplification and sequencing reactions were efficient in more than 60% of the HCV infected isolates, the HCV *NS5B* gene seems to be suitable for diagnostic. Indeed, the World Health Organization (WHO) reports show that genotypes 4, 5, and 6 most commonly occur in Africa, in Asia and in the Middle East. In this study, genotype 4 was identified and documented as belonging to subtype 4a. Subtypes 1a and 1b that are mostly found in developed countries and that require a longer duration of treatment to induce viral clearance compared to infections caused by genotype 2 and 3, also circulate in Cameroon, showing that HCV infection represents a serious threat in HIV infected people. WHO reports show that HCV genotypes 1a and 1b cause approximately 60% of hepatitis C infections worldwide, and are responsible for chronic hepatitis. In the United States for instance, genotypes 1a and 1b account for roughly 75% of hepatitis C infection. It is also reported that fewer people with genotype 1 infections less experience viral clearance with treatment compared to people with genotype 2.

In this study, no genetic link was observed between the HIV strains (CRF02_AG) and the HCV ones. This might be due to the fact that HIV and HCV viruses do not necessary infect the same type of cells. Although HCV viral replication has been reported in B cells, T cells, monocytes, macrophages, and other macrophage-like cells such as Kupffer cells and dendrocytes, HCV more commonly infects hepatocytes whereas HIV infects antigen presenting cells, and preferentially T lymphocytes.

Epidemiological data

Data collected and analyzed showed that 64.30% of HIV infected people were female and 35.70% were male. As a consequence, women were more infected than men. Therefore, the majority of HIV infected patients were young women, from 17 to 35 years, whereas the majority of HIV infected men were older from 26 to greater than 50 years. Above 50 years, 30% documented HIV/HCV co-infected patients were men, whereas only 7% of

woman were identified as HIV/HCV co-infected patients. Overall, HIV/HCV co-infected patients were mostly men, and older than HIV mono-infected patients who were mostly women. This study then confirms other research that demonstrated that HIV/HCV co-infected individuals are older than the HIV mono-infected ones, and that persons aged above 35 years have the highest HCV infection (Njouom *et al.*, 2003; Adoga et al., 2009). Globally, the present study confirms previous investigations demonstrating that HIV/HCV co-infected individuals are older than the HIV mono-infected patients. Actually, taking into account the biochemical markers (ASAT and ALAT) of liver function analysed in those patients, and CD4+/CD8+ count, data collected showed that serum liver enzyme activities (ASAT and ALAT) are significantly elevated in co-infected patients compared to mono-infected individuals, denoting that HIV/HCV co-infection is a risk factor for liver function. Furthermore, data show a low CD4+ T cells counts and a high CD8+ T cells counts in co-infected patients, and lower CD4+and CD8+ cells counts in immune-depressed patients (Djuidje Ngounoue, 2009).

CONCLUSION

Throughout the present study, it has been illustrated that HIV Circulating Recombinant Forms CRF02_AG are prevalent in Cameroon, while HCV strains belong to genotypes 1, 2, and 4 with 1a, 1b, 1c, 2a, 2c, 2k, and 4a as corresponding subtypes. HCV subtypes 1a and 1b were identified in Cameroon, with the inference that HCV infection represents an added threat to people living with HIV/AIDS, for it perturbs the liver function and negatively affects the immune system in co-infected patients. HIV/HCV co-infected individuals are older and more affected than the HIV mono-infected patients. Fundamentally, there is evidence of virulent hepatitis C virus in Cameroon and therefore an imperative for comprehensive molecular database build-up, and monitoring of the disease progression.

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