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Evaluation of HIV Viral Load Activity at the Bacteriology-Virology Laboratory in the University Hospital Center of Brazzaville

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Abstract

Introduction: The bacteriology-virology laboratory of the teaching university hospital of Brazzaville, was equipped with a real-time PCR device like Miniopticon (Biorad®, France). The aim of this work was to do an evaluation of the HIV viral load activity, with a view to proposing some recommendations. **Material and methods**: Retrospective study, January 2013 to March 2015, in patients on first line ARV three-therapy, pre-inclusion therapy checkup in HIV positive patients, but again screening after sexual abuse in women or accident of exposure (AES). A blood sample on EDTA tube was made and RNA extraction with Qiagen kit. Ultrasensitive HIV-RNA quantification was performed using the Generic HIV real-time PCR assay (Biocentric®, Bandol, France). **Results**: 126 patients were included. The mean age was 37.63 years +/− 10.43 years, sex ratio F/H = 2.3. The HIV viral load was detectable in 94 cases (74.6%). Concerning patients with detectable viral load (copies/ml): 403 to 996 in 35 cases (37.23%), 1411 to 1812 in 41 cases (43.62%) and >1814 in 5 cases (5.32%) (therapeutic failure). **Conclusion**: This work reports success in the setting up of the molecular biology unit. Procedures that implement information and education actions on the risks associated with AES must be disclosed.

Keywords

Viral Load, HIV, Miniopticon, CHU, Brazzaville

1. Introduction

The HIV epidemic has been rapidly spreading throughout the Republic of Congo. Based on a national survey performed in 2009, the prevalence was estimated to 3.2% of infected population [1]. AIDS-related immunosuppression leads to exposure of patients to opportunistic diseases such as tuberculosis [2]. People living with HIV/AIDS need antiretroviral (ARV) therapy. To control the epidemic, the biological monitoring of patients requires a biological assessment including HIV viral load [3]. Indeed, HIV viral load is recommended as the preferred monitoring approach to diagnose and confirm ARV treatment failure [4]. Until 2011, in Brazzaville, only the national public health laboratory was responsible for determining the viral load of HIV patients. In 2011, the bacteriology-virology laboratory of the teaching university hospital of Brazzaville, was equipped with a real-time PCR device like Miniopticon (Biorad®, France), in order to carry out the survey of people living with HIV/AIDS. Thus, despite some difficulties, relating to the acquisition of the necessary additional equipment, consumables, reagents and also the compartmentalization of the local, a molecular biology unit had been created within the laboratory and was functional in 2013. This unit includes a genetic material extraction room, a PCR mix preparation room, a genetic material depot room and a genetic material amplification room [5]. The aim of this work was to do an evaluation of the HIV viral load activity, with a view to proposing some recommendations.

2. Material and Methods

Study and patients: This was a retrospective and descriptive study, which looked at patients received at the bacteriology-virology laboratory of the teaching Hospital of Brazzaville, from January 2013 to March 2015. It was patients in routine consultation who consent to the study protocol.

The inclusion criteria were: 1) patients on first line ARV three therapy: zidovudine (AZT) + lamivudine (3TC) + Névirapine (NVP), 18 months or 36 months after the beginning of the treatment, 2) pre-inclusion therapy checkup in HIV positive patients 3) screening after sexual abuse in women or accident of exposure (AES) to blood in Occupational Blood Exposure (OBE) in hospital, at least a month of exposure.

The exclusion criteria were: HIV patients at the beginning of the first line ARV three therapy and patients who don't give the contentment to participate to the study.

HIV RNA extraction: A blood sample on EDTA tube was then made for determination of the viral load. The blood sample for the viral load was divided

into 3 aliquots of one millilitre of plasma: one millilitre of which was used for the determination of viral load and the other 2 frozen at −70˚C. The HIV RNA was extracted from 500 µl of plasma by using the QIAamp viral RNA minikit (Qiagen, Courtaboeuf, France) according to the manufacture's recommandations [6] [7].

Real time PCR: Ultrasensitive HIV-RNA quantification were performed using the Generic HIV real-time PCR assay (Biocentric®, Bandol, France) on the Miniopticon equipment (Biorad, France). The detection threshold of this method being 100 copies/ml. The Generic viral load HIV-1 test (Biocentric®, Bandol, France) is for determining viral load in patients infected with human immunodeficiency virus type 1 (HIV-1). It exploits the principle of RT-PCR by hydrolysis of a double-labeled nucleotide probe with a fluorescent reporter group (FAM™) and a 3' quensher (MGB) group, according to the Taqman technology. During PCR, sense and antisense primers are hybridized to a specific sequence at the level of the amplicons. The probe contained in the same reaction mixture hybridizes to a target sequence of the amplicon. When the probe is intact, the spatial proximity between the reporter and the quensher inhibits the fluorescence of the reporter, mainly by a Föster-type energy transfer. During PCR, the probe specifically binds between the two sites where sense and antisense primers are hybridized and inhibits any Taq polymerase activity, but activates its 5'-3' exonuclease function which cleaves the probe between the reporter and the quensher. The reporter released from the quensher emits a fluorescent signal, recorded in real time by sensors. Thus, freed of the probe fragments, the target sequence can be read and polymerized by Taq polymerase. The increase in the fluorescence signal is detected only if the target sequence is complementary to the probe and if it is amplified during the PCR. Thus non-specific amplification cannot be detected. Regarding to this reaction principle, the increase in fluorescence is directly proportional to the amplification of the target during the PCR. The evolution of the amplification is represented by a sigmoidal curve, which can be divided into two phases: a phase corresponding to an exponential amplification during which the quantity of PCR product obtained at each moment is directly function of the number of initial copies, at the beginning of the exponential amplification phase, the moment when the signal comes out of the background noise corresponds to a certain number of cycles, called Ct (Cycle threshold). The exponential amplification is followed by a plateau phase which corresponds to a slowing of the amplification, due to the depletion of the reagents. Thus, the results are expressed in number of copies per milliliter (copies/ml) [8].

The method included in first time reverse transcriptase followed by the amplification of the DNAc. The PCR reactions were carried out in a 48 white plate in a volume of 20 µl containing 0.5 µl of primer A, 0.5 µl of primer B, 0.5 µl of probe C, 3.5 µl of H_2O, 5 µl of Mix enzymatique 4× and 10 µl of RNA sample extract.PCR amplification comprised an initial reverse transcription step at 50˚C for 10 min, an activation enzyme step at 95˚C for 5 min, a denaturation step at

95˚C at 15 secondes, a hybridization step at 60˚C cycle for 1 minute followed by 50 amplification cycles.

Statistical analysis: Data entry and analysis was done using Excel 2007 and Epi-Info 7 softwares. The results were expressed as averages for the quantitative variables and as a proportion for the qualitative variables.

3. Results

During the study period, 126 patients were included. The mean age was 37.63 years +/− 10.43 years with extremes of 10 years and 65 years. It was noted that 38 patients (30.16%) were male and 88 patients (69.84%) female (sex ratio F/H = 2.3).

The HIV viral load was detectable in 94 cases (74.6%) and undetectable in 32 cases (25.4%).

The Distribution of patients with undetectable viral load is noted in Table 1. Table 2 reported the distribution of patients with detectable viral load according to the results of viral load. Table 3 reported the categories of patients with detectable viral load and then, concerning 8 antiretroviral therapy experienced patients, their viral load was between 1008 to 1409 copies/ml.

Table 1. Distribution of patients with undetectable viral load.

Category of patients	n	%
Patients on antiretroviral therapy	19	59.4
Accident of exposure to blood (AES)	10	31.2
Screening after sexual abuse	03	9.4
Total	**32**	**100**

Table 2. Distribution of patients with detectable viral load according to the results of viral load.

Viral load (copies/ml)	n	%
105 - 395	5	5.32
403 to 996	35	37.23
1008 to 1409	8	8.51
1411 to 1812	41	43.62
>1814	5	5.32
Total	**94**	**100**

Table 3. Categories of patients with detectable viral load.

Categories of patients	n	%
Inclusion in the antiretroviral therapy protocol	89	94.7
Patients on antiretroviral therapy	5	5.3
Total	**94**	**100**

4. Discussion

This work presents the preliminary results concerning the determination of HIV viral load, in the bacteriology-virology laboratory of the teaching hospital in Brazzaville. Three categories of patients have been identified: HIV patients on antiretroviral therapy or before ARV therapy inclusion, accident of exposure to blood in occupational blood exposure, sexual abuse in women. In this study, 126 patients were recruited. This number is low compared to one of patients normally on antiretroviral therapy, during the study, who should use viral load, except of the new cases. Indeed, 3.2% of the positive HIV population [1] was noted in Congo. This should correspond to nearly 96,000 to 128,000 people out of nearly 3 to 4 million of the general population throughout the country. In addition, WHO reported in 2017, 1.7 million new cases in sub-Saharan Africa [9]. This explains that during the study, the number of patients should be greater than 96,000 to 128,000 people. Several reasons may explain this low participation: the prior existence of the national public health laboratory, where patients are referred before the setting up of the molecular biology unit at the CHU of Brazzaville, ruptures in reagents each year not renewed immediately by the National AIDS Control Program, all patients followed by clinicians are not referred to the laboratories for the purpose of determination of the viral load.

One limit of the study was represented by the HIV viral load threshold quantification fixed at 100 cp/ml. Indeed, it is recommended to use a PCR method for which the threshold is set around 50 copies/ml [8] [10].

Regarding accidents of exposure to blood and screening after sex abuse, they represent the situations that may lead to health care workers and victims of rape, infections caused by pathogens such as the hepatitis B virus, the hepatitis virus C and HIV. Indeed, a study in Ivory Coast showed a prevalence of AES near health staff of 60% [11] and 54% in Central African Republic [12]. Another study showed a prevalence of 0.4% of gynecological admissions, of sexual abuse patients, with two HIV-positive patients [13]. Similarly, Mbassa and Ngoh report HIV seroprevalence of 37.5% among victims of sexual abuse in Cameroon [14]. In our study, there was 31.25% of patients admitted for AES in Occupational Blood Exosure (OBE). Management was carried out by infectious physicians. Unfortunately, the hygiene procedures in case of AES are not popularized, the practitionners victims of OBE do not apply them in time and consult a little late infectious physicians. Hence the need for pre-treatment screening, using PCR which is a method to detect the presence of the virus in the body rather quickly compared to viral serology. These results show the interest of the hospitals in Congo in general and the CHU of Brazzaville in particular, to put in place the necessary procedures allowing the hospital practitioners, to react quickly against the OBE, with a view to fight against the HIV infection and other infections transmitted by blood. Similarly, the program for the care of persons who have been sexually abused needs to be strengthened and the procedures for the care of these persons divulged to the general population, in order to facilitate the fight

against STIs, including HIV.

This study shows the interest of the molecular biology laboratory in the monitoring of people living with HIV. For this purpose, viral load was determined in patients before inclusion of antiretroviral therapy in 89 cases (94.7%). The development of HIV strategies in underdeveloped countries and in particular in Congo should be encouraged, so that all screened patients must have the opportunity to obtain an initial viral load before inclusion.

For patients on first-line antiretroviral therapy, namely, zidovudine (AZT) + lamivudine (3TC) + nevirapine (NVP), the viral load was undetectable in 59.37% of cases in patients after 18 months of treatment. This shows a certain therapeutic efficacy of this therapeutic line, which remains to be improved because the 90-90-90 UN-AIDS initiative provides 90% viral load undetectability in person living with HIV now until 2020 [15].

Furthermore, it was noted that 36 months after the beginning of the treatment, antiretroviral therapy experienced, 5 (5.3%) patients, have viral load between 1008 to 1409 copies/ml (>1000 copies/ml), after two analyses. This can representing virological failure to first-line drugs. This result is low compared to a study reporting 41.7% resistance in Burkina Faso in a population of ART patients [16]. Similarly, in 2009 in Central Africa, Charpentier and colleagues reported that one-third (34%) of children receiving 1st-line regimen (median of treatment = 18 months) was in virological failure with selection of drug resistance mutations (DRMs). Then, in 40%, which was associated in 85% of cases with viruses harboring at least one drug-resistance mutation to nucleoside reverse transcriptase inhibitors (NRTI) or nonnucleoside reverse transcriptase inhibitors (NNRTI), and in 36% of cases with at least one major drug-resistance mutation to NRTI or NNRTI when excluding the M184V mutation, and therefore eligible to 2nd-linetreatment [17]. In our case, sequencing of HIV-1 resistance genes was recommended in order to determine the resistance genotypes and thus to monitor therapeutic switches.

5. Conclusion

Regarding the WHO recommendations, the determination of HIV viral load in the follow-up of people living with HIV in this work is an important parameter that has made it possible to determine the therapeutic efficacy and probable development of cases of failure to first-line treatment. Despite the difficulties encountered, this work reports success in the setting up of the molecular biology unit at the bacteriology-virology laboratory of the University Hospital of Brazzaville, through the determination of HIV viral load. This study challenges hospital practitioners and health authorities to strengthen, disclose procedures and implement information and education actions on the risks associated with AES. Similarly, an improvement is to be made in the sensiblization of doctors for a systematic demand for viral load in the follow-up of patients living with HIV, as well as the national AIDS program, in order to perpetuate supply of the laboratory in reagents.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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