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Contribution of Nested PCR for the Diagnosis of the Immunodeficiency Virus and the Detection of Endogenization of the Provirus in Serodiscordant Couples in Kinshasa, Democratic Republic of the Congo: A Preliminary Diagnostic Study

Cagod B. Inkale[1,2,3*], Isaac Woto[2,3], Alain K. K. Ilunga[2,3,4], Charlotte L. Tshinguta[2,5], Tarcisse Kilara[2], Gisele Kabengele[2], Christian Tshilenge[2], Berry I. Bongenya[2,3,6], Erick N. Kamangu[2,3*]

[1]Makala General Reference Hospital, Kinshasa, Democratic Republic of the Congo
[2]Department of Molecular Biology, Faculty of Medicine, University of Kinshasa, Kinshasa, Democratic Republic of the Congo
[3]"HIV/AIDS Focus" Research Group, Kinshasa, Democratic Republic of the Congo
[4]Institute of Research in Health Sciences, Kinshasa, Democratic Republic of the Congo
[5]Kinshasa Veterinary Laboratory, Kinshasa, Democratic Republic of the Congo
[6]Kinshasa Provincial Reference General Hospital, Kinshasa, Democratic Republic of the Congo
Email: *cagodinkale@gmail.com, *erick.kamangu@unikin.ac.cd

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Abstract

Context: The Polymerase Chain Reaction (PCR) amplification technique has aroused and continues to arouse great enthusiasm in the biomedical sciences. It has enabled the development of ultra-sensitive tests for HIV, thanks to which it is possible to diagnose this infection early, even before the appearance of specific antibodies. **Objective:** Confirm the molecular diagnosis of HIV in serodiscordant couples followed in Kinshasa and find the presence of the viral genome in seronegative partners. **Methods:** A cross-sectional diagnostic study was conducted in the city province of Kinshasa in two different health structures. Sociodemographic information as well as information on the results of the various tests carried out was noted on a data collection sheet for statistical analysis. **Results:** In total, 36 (thirty-six) samples were analyzed by Classic Nested PCR. 18 positive samples were confirmed HIV positive and the remaining 18 negative samples were confirmed HIV negative. **Conclusion:** Nested PCR remains the reference test for the diagnosis of virological diseases, given its very high sensitivity and specificity.

Subject Areas

Diagnostics

Keywords

Serodiscordant Couples, HIV, Nested PCR, Kinshasa

1. Introduction

Polymerase chain reaction or polymerase chain reaction is a high sensitivity molecular biology technique that detects DNA and RNA fragments of viruses, bacteria and other microorganisms [1].

Its principle is attractive since the shortcomings of serological methods can be linked to the mode of replication of the virus, with a possible eclipse phase and above all the need for integration into the cellular genome without the virus or its antigens being expressed necessarily continuously [1].

PCR has enabled the development of ultra-sensitive tests for the Human Immunodeficiency Virus, thanks to which it is possible to diagnose this infection early, even before the appearance of specific antibodies. But in addition to the relative technical ease of execution, there are difficulties of interpretation and training of the appropriate personnel [2] [3].

In the context of this study, Nested PCR is a variant of classic PCR which is more specific than simple Classic PCR. It allowed us to arbitrate, to decide on the seropositivity of partners in so-called HIV serodiscordant couples.

So-called HIV-negative people living in a couple with people living with HIV, presenting mechanisms of resistance to HIV; can be people carrying the virus, transmitting the virus but not developing AIDS.

These people have inherited or acquired a mutation that gives them protection against HIV/AIDS. And thanks to molecular biology, scientists have been able to prove or highlight these resistance mechanisms. It was important to be able to accurately analyze the deoxyribonucleic acid (DNA) of seronegative partners to highlight the endogenization mechanism of the provirus. Exogenous retroviruses are transmitted horizontally between host individuals, with disease outcome depending on interactions between the retrovirus and the host organism. This mechanism allows the virus to stay as long as possible in the human genome without causing disease. And its detection is not possible with techniques using antigens and antibodies as biological diagnostic material for HIV.

In this study, the objective was to confirm the presence of HIV in discordant couples who are being followed for HIV/AIDS in Kinshasa, and to investigate the phenomenon of endogenization of the provirus in seronegative individuals using the PCR DNA test, specifically the Classic Nested method. The aim is to improve screening, management, and follow-up of patients.

2. Methods

2.1. Setting, Sampling and Study Design

It is a cross-sectional, descriptive study with a diagnostic aim which was carried out in the city of Kinshasa in the Democratic Republic of the Congo. The samples were taken from different randomly chosen health centers for PLHIV in two different districts of our choice, but all the hospital structures are in the Urbano-rural health zone. Among the structures randomly chosen for their proximity to the investigators, the Monkole Hospital Center is located in the Lukunga district of the Mont-Ngafula commune within the Mont-Ngafula II health zone. The Sainte Anne Health Center, on the other hand, is situated in the La Funa district of the Selembao commune within the Selembao health zone.

The size of the population studied was not determined upstream; the sampling used was that of non-probability convenience.

For this preliminary study, the inclusion period extended from November 2021 to August 2022.

2.2. The Study Population and Inclusion Criteria

Out of eighteen couples included, 14couples came from CH Monkole out of a total of 28 serodiscordant couples followed and 4 couples came from CS Sainte Anne out of a total of 9 serodiscordant couples followed. The discordant couples had been followed up on a regular basis at the care centres. The inclusion criterion referred to any person in a couple living with HIV whose spouse or spouse is HIV-negative and both of whom are followed in the same care center and present at the time of the survey.

2.3. Statistical Analysis of Data

The information was recorded on the survey sheets, and entered on the Windows Excel software. Statistical data analyzes were performed on Statistical Package for the Social sciences (SPSS) version 20.0 for Windows.

2.4. Blood Samples

A minimum of 5.0 ml of blood was collected in the tubes containing EDTA from the respective testing centers by the survey team. The blood collected was transferred to the Molecular Biology Laboratory in compliance with the cold chain (conservation 5˚C to 10˚C). An aliquot of around 2 µL of whole blood was spread on filter paper stored at –20˚C in the Molecular Biology laboratory for subsequent analyses.

2.5. DNA Extraction and Amplification

The genetic material extraction was performed at the Molecular Biology Laboratory of the Faculty of Medicine of the University of Kinshasa, in the Democratic Republic of the Congo. Whole blood was smeared and dried on blotting paper before using the QIAamp DNA Mini Kit from QIAGEN for DNA extraction.

The extracted DNA was stored at −20˚C and quantified using a NanoDrop 2000 Spectrophotometer at the Human Genetics Laboratory of the Faculty of Medicine of the University of Kinshasa.

After ensuring that the DNA had actually been extracted, classical gag and Pol PCR and Nested gag and pol PCR were run to determine the presence of proviral DNA. A 3rd Nested PCR env in case of discordance of the results of gag and pol.

The primers for the first step of the HIV PCR consisted of the following nucleotides: GAG1 (GCA-TTA-TCA-GAA-GGA-GCC-ACC-CCA-CA) and GAG4 (TCC-TGA-ACG-GTA-CTA-GTA-GTT-CCT-GCT-A) for gag amplification, POLITG1 (CCC-TAC-AAT-CCC-CAA-AGT-CAA-GG) and POLITG4 (TAC-TGC-CCC-TTC-ACC-TTT-CAA) for pol. Primers for Nested PCR were: GAG2 (AGT-GGG-GGG-ACA-TCA-AGC-AGC-CAT-GGCA-AAT) and GAG3 (TGC-TAT-GTC-ACT-TCC-CCT-TTG-TTC-TCT-C) for gag. POLITG2 (TAA-GAC-AGC-AGT-ACA-AAT-GGC-AG) and POLITG3 (GCT-GTC-CCT-GTA-ATA-AAC-CCG) for pol. The different PCRs were carried out under the conditions described in Table 1. The primers were ordered through the firm Erogentec.

2.6. Interpretation of Results by Electrophoresis

The revelation of the amplification of the amplified fragment (Nested gag and pol) under UV after electrophoretic migration at 80 volts, 300 Amp for 1 hour on a 1% agarose gel in 1 × TAE (Tris/Acetate/EDTA buffer) in the presence of GelRed.

For a sample to be counted positive for HIV, the amplified fragments (Nested gag and Pol) must migrate at 141 bp and 147 bp.

2.7. Ethical Consideration

Participation in this work was voluntary. All the couples had previously signed a free and informed consent, the survey form was drawn up in French, the official language, and in Lingala, the spoken language of Kinshasa people. Respect for human life and patient confidentiality were respected in accordance with the standards of Good Clinical Practice. The blood samples were collected by technicians from the selected centers under the supervision of the principal investigator and the heads of the centers' HIV units. All procedures pertaining to this work were closely monitored by the work team.

This study had received the approval of the Ethics and Scientific Committee of the Kinshasa School of Public Health (Ref: ESP/CE/115/2021). We had also obtained the agreement of the managers of the centers in which we worked.

All results and individual patient information are kept confidential. The only people who have been able to consult the results are the members of the scientific team and the managers of the various centers. The results were coded by the principal investigator and carried no center identifiers.

Table 1. PCR temperature cycle.

	Gag and pol	Nested pol and gag
Denaturation initial	95˚C: 9 min	95˚C: 9 min
Denaturation per round	94˚C: 1 min	94˚C: 1 min
Hybridization Per round	50˚C: 1 min	50˚C: 1 min
Elongation Per round	72˚C: 1 min	72˚C: 1 min
Elongation Final	72˚C: 10 min	72˚C: 10 min
hold	4˚C	4˚C
number of cycle	40	40

3. Results

Eighteen (18) couples were being monitored at care centers for HIV serodiscordance after having tested positive for some and negative for others on RDTs. They included in this study to establish the diagnosis from a molecular point of view.

3.1. Confirmation of the Molecular Diagnosis of Serodiscordant Couples

A total of 36 samples were taken, intended for analysis at the Molecular Biology and Human Genetics laboratories.

After extraction of the human genomic DNA, the extracted DNA is quantified with a nanodrop 2000 to be reassured of its purity. The samples were divided into 2 groups, that of the seronegative and that of the seropositive. In each group a median was calculated. In both tables of the calculated median, the extracted DNA was of good quality but of low concentration (Table 2).

Out of 18 samples from HIV-positive individuals analyzed, all were found to be positive by Classic Nested PCR (Table 3 and Figure 1).

Out of 18 samples from seronegative individuals analyzed, all were found to be negative by Classic Nested PCR (Table 3 and Figure 2).

3.2. Revealing the Mechanism of Endogenization of HIV in HIV-Negative Partners

Table 4 reveals that out of 18 partners declared negative to the RDTs are also negative to the Nested PCR therefore the sequences sought from the viral genome are absent in the human genome of the seronegative partners.

4. Discussion

This study consisted in confirming the presence of HIV in serodiscordant couples followed in Kinshasa. After analyzing the results, the discussion revolves around the following points.

Table 2. Median values of partner DNA quantification.

Median	DNA concentration (ng/µL)	Ratio 260/280	Ratio 260/230
Seronegative	**7.15**	**1.95**	**0.165**
HIV positive	**12.25**	**1.95**	**0.17**

Table 3. Confirmation of the molecular diagnosis of HIV in HIV serodiscordant couples followed in care centers by Classic Nested PCR.

Serological/molecular analysis	Nested Gag	nested pol
18 HIV positive people on RDTs*	**18**	**18**
18 HIV-negative people on RDTs*	**18**	**18**
Total	**36**	**36**

*Determine and uni-gold.

Table 4. Determination of the presence of the viral genome in seronegative partners.

Molecular Analysis	Nested Gag	Nested pol
18 HIV-negative partners on RDTs*	**0**	**0**

*Determine and uni-gold.

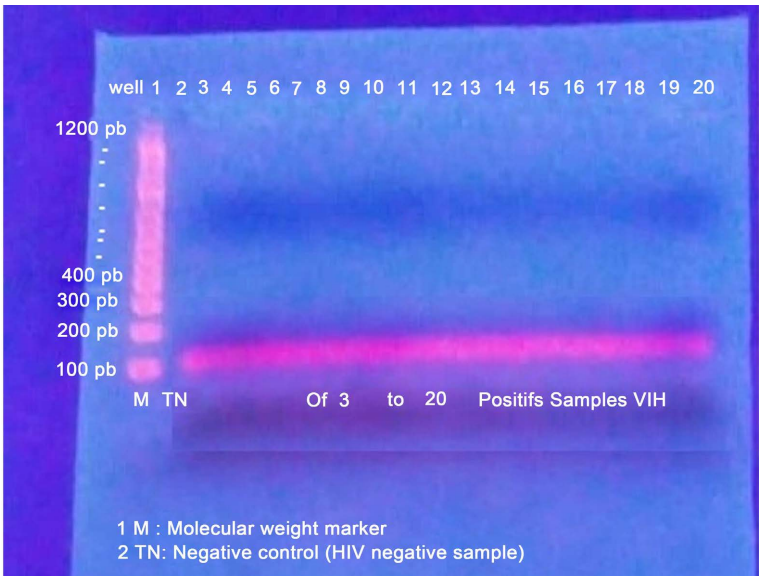

Figure 1. Visualization on electrophoresis of fragments amplified by Nested PCR in HIV-positive partners.

4.1. Confirmation of the Molecular Diagnosis of HIV in Serodiscordant Couples Followed at Care Centers

4.1.1. Interpretation of DNA Quantification Results at Nanodrop 2000

The medians calculated for the DNA concentration and the 260/280 ratio for both partners (HIV positive and HIV negative) corroborate with previous studies that outline the backbone of the theory.

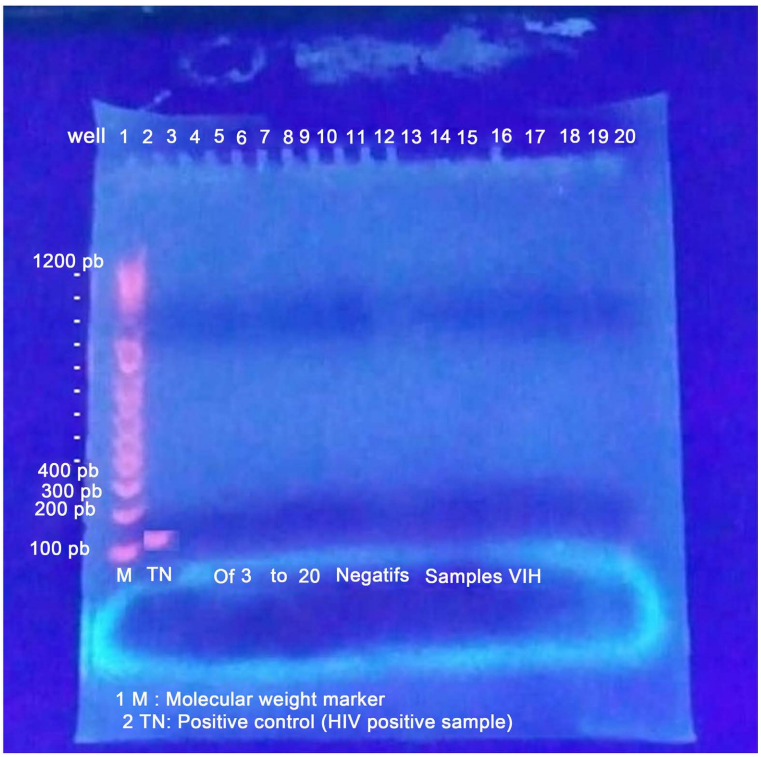

Figure 2. Visualization on electrophoresis of fragments amplified by Nested PCR in HIV-negative partners.

Compared to the normal range of the A260/280 nm ratio which is 1.8 to 2.0 for DNA [4], the found value of the median ratio is the norms. A study carried out in the Molecular Biology Laboratory of the UNIKIN Faculty of Medicine in 2019 [5] its results on the quality of the extracted DNA corroborate with the value found at the 260/280 ratio. Especially since the author had used the Qiagen kit.

But according to a study conducted by the Biological Resource Center in France in 2015, the acceptable interval for the A260/280 nm ratio is between 1.6 and 2.2 using the Qiagen kit [6]. Considering this range of values, all the samples we used give acceptable values of the A260/280 nm ratio. As for the 260/230 absorbance, the values found are less than 1.8; therefore our results were probably contaminated by ethanol, because it is cited among the reagents absorbing at 230.

The DNA concentration values found are low compared to the recommendation (50 ng/µL) [4].

4.1.2. Interpretation of Classical Nested PCR Results in HIV Serodiscordant Couples

36 samples were amplified by Classic Nested PCR, after migration to electrophoresis, 18 samples from seronegative people were confirmed negative by PCR and 18 other samples from seropositive people were also confirmed positive by PCR.

The analysis confirmed the results issued by the two care centres. Determine and uni-gold are tests used in the DRC for the definitive diagnosis of HIV. The first test is antigenic, it is designed to detect the P24 Ag of HIV-1 and 2 and the following test, it is designed to detect anti HIV-1 and 2 Ab. This synergy of two successive tests exposes the presence of HIV in the blood stream. But for these tests to be highly sensitive, PLHIV must be or completely exceed the period of primary infection. The RDTs provide many cases of indeterminate and also false positives not only according to the quality of the test but also according to the replicative cycle of HIV. The molecular test, Nested PCR, is a highly sensitive test, given its method of implementation.

A few studies before us have carried out this exercise of diagnostic comparison or confirmation in patients who have undergone RDTs. Two studies published in the journal Open access: the one published in 2014 on 171 samples collected, 107 positive, 43 negative and 21 indeterminate by RDTs. After the classic Nested DNA PCR, 112 samples were positive and 59 negative. The 21 samples indeterminate by the RDTs, 3 were positive and 18 negative by the PCR [7] and that published in 2020 out of 100 samples collected, 60 were positive and 40 negative for HIV by the RDTs and after amplification 65 samples were found to be positive and 35 samples negative to the molecular test [8].

All these published studies and ours show to what extent Nested PCR remains the reference test in the arbitration of results in people supposedly infected with HIV.

4.2. On the Determination of the Presence of the Viral Genome in Seronegative Partners

Regarding the presence of sequences of the viral genome in the DNA of seronegative partners to elucidate the phenomenon of endogenization of HIV, after amplification with a conventional thermocycler, on reading the gel, we noted the absence of viral sequences in their DNA. We dare to believe that the resistance mechanism that makes these people negative for HIV, and not developing the disease, is outside the cell nucleus. In the future, other extra and cytoplasmic resistance mechanisms will be explored to find said resistance.

5. Conclusion

Serodiscordance in couples is a very real fact, proven in various analyses that we have had couples undergo. The Classic Nested PCR remains a reference test in the case of diagnostic ambiguity, especially that of HIV; it validates and gives reliable results.

Conflicts of Interest

The authors declare no conflict of interest regarding the publication of this article.

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Abbreviations

AIDS: Acquired Immune Deficiency Syndrome; **DNA:** Deoxyribo Nucleic Acid; **DRC:** Democratic Republic of the Congo; **HIV:** Human Immunodeficiency Virus; **PCR:** Polymerase Chain Reaction; **RDT:** Rapid Diagnostic Test; **UNIKIN:** University of Kinshasa.