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Research Article
Molecular Evaluation of the Efficacy of the AZT+3TC+NVP Therapeutic Regimen in the Treatment of HIV-1 Patients in Senegal

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Abstract

Background and Objective: Antiretroviral treatment is a powerful means of reducing the risk of HIV transmission. Several observational studies have demonstrated a reduction in the risk of sexual transmission of HIV in people on ARV treatment and the main objective of antiretroviral treatment is to prevent progression to AIDS and death. Thus, the objective of this study was to evaluate the efficacy of the AZT+3TC+NVP therapeutic regimen in the treatment of HIV infection. **Materials and Methods:** This is a retrospective study of the treatment of HIV-1 seropositive patients. During this study, 445 patients were included and followed in the molecular biology laboratory of the HIV/AIDS program from 2014 to 2021. All plasma samples were from HIV-1-positive patients. Plasma viral load tests were performed on Abbott Real-Time HIV-1® (m2000sp/rt) and COBAS® AmpliPrep TaqMan® (Roche) v.2.0. As $p<0.05$ was considered statistically significant. **Results:** The median age was 33 years, women represented 71.2% of patients. Viral load was undetectable in 6.7% and 73.5% of patients at 6 and 12 months, respectively. At 12 months, virological suppression was 80.5% in women and 56.3% in men ($p=0.01$). At 12 months, patients aged had significantly greater viral suppression than the youngest ($p=0.001$). At the 18 months, 80.2% of patients had a suppressed viral load and 13% of patients had virologic failure. **Conclusion:** The AZT+3TC+NVP was effective in terms of virological suppression. Viral suppression was associated with sex and age at 12 months of treatment. Therapeutic failure was relatively low. This treatment is still effective and less expensive, which is why it is so interesting for countries with limited resources.

Key words: HIV-1, antiretroviral-therapy, sub-sahara, viral load, AZT+3TC+NVP

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The HIV infection remains a public health problem worldwide. The use of highly effective and well-tolerated ARVs that patients can easily adhere to and that provide long-lasting viral suppression remains the ultimate goal of antiretroviral therapy against HIV infection. The aim of antiretroviral therapy is to reduce the viral load to undetectable levels and so as to achieve the greatest immunological recovery and reduction of clinical progression allowing better immune restoration and mortality from HIV infection[1]. Thus gradual decrease in prevalence and new infections is possible thanks to free access to Antiretroviral (ARV) treatments in several regions and the availability of triple antiretroviral therapy (ART).

Antiretroviral therapy should make the patient's viral load undetectable (<50 copies mL^{-1}), promoting immune restoration and reducing the risk of viral drug resistance and HIV-associated clinical events[2]. Early initiation of antiretroviral therapy leads to a rapid drop in viral load, which reduces the risk of HIV transmission[3].

Several studies showing the limits of CD4 measurement in the management of HIV-positive patients have made viral load the main prognostic marker for evolution and therapeutic follow-up[4]. The measurement of the plasma viral load makes it possible to evaluate the progression of the infection, the effectiveness of the antiretroviral treatment and the appearance of resistant genotypes of HIV. Inaccurate viral load measurement can lead to inappropriate patient management.

Genetic variability and instability can lead to HIV resistance to ARVs and cause treatment failures. This genetic variability results from the error rate of nucleotide incorporation by reverse transcriptase, during the reverse transcription of viral RNA into DNA, the incidence of genetic mutation rate is 1 to 10 per genome. Initial antiretroviral therapy used in national HIV/AIDS programs in resource-limited settings included Nucleoside Reverse Transcriptase Inhibitor (NRTIs: AZT, TDF, 3TC and d4T) and Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI: NVP and EFV)[5-7].

Given the link between HIV genomic variability and therapeutic resistance, the study of the efficacy of ART regimens is necessary to give recommendations to national AIDS control programs and clinicians in order to minimize treatment failures.

Virological failure in HIV-1-infected patients can be associated with the emergence of Antiretroviral (ARV) drug resistance, narrowing options for future therapy[8]. Although the genetic barrier to resistance describes the threshold of mutations required for clinically meaningful loss of drug susceptibility[9], the emergence of resistance can also be influenced by the drug's structure[10], inhibitory quotient[11] and pharmacokinetic forgiveness[12]. The cornerstone of durable suppression of HIV replication is the maintenance of a potent and tolerable regimen to which the patient can adhere. Adherence is necessary to prevent the emergence and replication of drug-resistant strains of the virus[13].

It is therefore important to update ART combinations, especially in regions with limited resources, to achieve the ending of AIDS in 2030.

To have an effective triple therapy allowing successful ART treatment, to limit treatment failures and HIV-related deaths, it is important to evaluate the ART regimens available.

In this context, the main objective of this study was to evaluate the therapeutic efficacy of zidovudine in combination with lamivudine and nevirapine (AZT+3TC+NVP) in Senegalese HIV-1 patients.

MATERIALS AND METHODS

Study population: A retrospective follow-up study of HIV-1 patients was conducted. During this study, 445 patients were included. Seropositive plasma samples were collected at the Molecular Biology Laboratory of the AIDS Program of the Senegalese Armed Forces at the Ouakam Military Hospital in Dakar (Senegal) from 2014 to 2021. All plasma samples were from HIV-1-positive patients. Consent was not required for these patients as plasma VL was performed as part of their clinical follow-up. The evaluation of the AZT+3TC+NVP combination was carried out from the initiation of treatment until the 18th month (from M0 to M18) with a six-monthly or annual follow-up. To be eligible, HIV-1-positive patients had to have been receiving the AZT+3TC+NVP treatment regimen for at least 6 months.

Sample collection: Whole blood was collected in 5 mL^{-1} BD K2E (EDTA) tubes (ref 368861) (Becton Dickinson, New Jersey, USA). After centrifugation at 6000 rpm for 20 minutes at 4°C, two aliquots of plasma were prepared for each patient, one for testing on Roche or Abbott and the other in reserve, immediately frozen at -80°C until testing. For each sample taken, an analysis report was submitted to each patient with the patient's identifier, age, sex, patient's HIV status, duration of ART and virological data.

HIV viral load measurement techniques: Each plasma sample was processed on either Abbott (m2000sp/m2000rt) or Roche (COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 v2.0) for HIV-1 RNA quantification.

Abbott real time HIV-1® (m2000sp/rt): The Abbott Test (m2000sp/m2000sp) is a real-time reverse transcriptase PCR Test for the quantitative determination of HIV-1 RNA in HIV-1 positive plasma. Extraction is done using 0.6 mL^{-1} of plasma and reverse transcriptase is followed by real-time amplification and detection of a fragment of the integrase region of the pol gene (pol/IN) of the genome of the HIV-1 with the m2000rt fluorescent probe test kit[14]. The Abbott platform detects the majority of HIV-1 M variants, A-H subtypes and CRFs such as CRF01-AE and CRF02-AG and also N and O divergent groups, in a range of linearity ranging from 40 to 107 copies mL^{-1}. Plasma samples are tested in the m2000sp/m2000rt instrument according to the manufacturer's instructions. The Abbott instrument is a closed automation system combining extraction, reverse transcriptase, PCR and real-time detection, reducing the risk of contamination. Each series of tests includes three controls (one negative, one strong positive and one weak positive). The analyzer automatically validates the manipulation and determines the presence or absence of HIV-1 nucleic acids according to a threshold cycle value (Ct value) which corresponds to the PCR cycle from which the signal detected indicates the presence of the amplicons. The analyzer automatically validates the manipulation and determines the presence or absence of HIV-1 nucleic acids according to a threshold cycle value (Ct value) which corresponds to the PCR cycle from which the signal detected indicates the presence of the amplicons.

Roche COBAS® AmpliPrep/TaqMan: The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 v2.0 (Roche Molecular Systems, Inc., New Jersey, USA) is a real-time reverse transcriptase PCR test. The extraction is done using the COBAS® AmpliPrep, using 1 mL of plasma according to the manufacturer's protocol. Next, reverse transcriptase is initiated automatically, followed by *in vitro* amplification and simultaneous detection of the highly conserved region of the Gag gene and the LTR (long terminal repeat) region of the HIV-1 genome using a TaqMan fluorescent probe (COBAS® TaqMan® 96). This test quantifies RNA over a range of 20-10,000,000 (1.3-7 log_{10}) copies mL^{-1}[15]. Plasma samples are tested in the Roche CAP/CTM96 instrument according to the manufacturer's instructions. The CAP/CTM instrument is a closed automation. The Abbott instrument is a closed automation system combining extraction, reverse transcriptase, PCR and real-time detection, reducing the risk of contamination. Each series of tests includes three controls (one negative, one strong positive and one weak positive). The analyzer automatically validates the manipulation and determines the presence or absence of HIV-1 nucleic acids according to a threshold cycle value (Ct value) which corresponds to the PCR cycle from which the signal detected indicates the presence of the amplicons. The quantification of VL using the Roche system was subject to an external quality assessment in 2018 by the College of American Pathologists (CAP) which deemed the results reliable.

Statistical analysis: Data acquisition and analysis were performed using Excel 2013 and SPSS version 21 software. Statistical cross-referencing was used for data comparison using the Chi-square Test for proportions and Fisher's exact Test for dichotomous variables with a theoretical significance level of 5% ($p<0.05$), considered statistically significant for all comparisons between groups.

RESULTS

Study population characteristics: Of 604 treatment-naïve patients, there were 445 met the inclusion criteria and 159 were excluded due to death, referral and loss of follow-up. The median age was 33 years with extremes of 1 year and 70 years and the majority of patients were aged 25 to 45 years (39.8%). Women represented 71.2% of patients and men 28.8% i.e., a sex ratio M/F=0.40.

Treatment efficacy: At the end of the 18-month study period, 80.2% of patients had a suppressed viral load, 6.7% had a decreased viral load and 13% of patients had virological failure. Viral load was undetectable in 6.7% and 73.5% of patients at 6 and 12 months, respectively. No undetectable viral load had been obtained over 18 months of ART (Table 1). As 86.9% had a viral load of less than 1000 copies mL^{-1} at 18 months of treatment.

Treatment success according to patient gender: At 6 months of AZT+3TC+NVP ART, the viral load was undetectable in 9.4% of men and 5.7% of women with a non-significant difference ($p=0.1$). At 12 months, virological suppression was greater in women than in men (80.5 vs 56.3%) ($p=0.01$). After 18 months, men had higher virologic failure (CV>1000 copies mL^{-1}) than women, 20.3 vs 10.1% ($p=0.00$, Table 1). Male patients had more virological evolution than female patients, 14.1 vs 3.8% ($p=0.001$).

Efficacy of treatment according to patient age: At 6 months of ART, viral load suppression was 9.41, 6.78 and 2.19%, respectively for patients in the age groups (0-25), (25-45), (45-65) years, ($p=0.2$). Patients over 65 years of age did not show viral suppression at 6 months of ART. At 12 months,

Table 1: Treatment success according to patient gender

Virological monitoring	Men 128 (%)	Women 317 (%)	Total population 445 (%)	p-value
Undetectable at 6 months	12 (9.4)	18 (5.7)	30 (6.7)	0.1
Undetectable at 12 months	72 (56.3)	255 (80.5)	327 (73.5)	0.001
Undetectable at 18 months	0	0	0	-
Virological evolution	18 (14.1)	12 (3.8)	12 (6.7)	0.001
Virological failure	26 (20.3)	32 (10.1)	32 (13)	0.001

Table 2: Treatment success according to patient age

Age, years, (N)	6 months	12 months	18 months	Virological evolution	Virological failure
(0-25)(170)	16 (9.41%)	94 (55.29%)	0%	21 (12.35%)	39 (22.94%)
(25-45)(177)	12 (6.78%)	145 (81.92%)	0%	6 (3.39%)	14 (7.90%)
(45-65)(91)	2 (2.19%)	81 (89.01%)	0%	3 (3.29%)	5 (5.49%)
>65(7)	0%	7 (100%)	0%	0%	0%
p-value	0.2	0.001	-	0.001	0.001

N = Patients by age group

ART success increased with patient's age. Indeed, the viral load was undetectable at 100, 89.01, 81.92 and 55.29%, respectively in patients aged over 65, (45-65) years, (25-45) years, (0-25) years (p= 0.001, Table 2). After 18 months of ART, 12.35% of patients aged 0 to 25 had a good virological evolution (decrease in viral load). The virological evolution was weaker in patients of the age groups (25-45) years, (45-65) years with 3.39 and 3.29%, respectively. Virological failure was significantly higher in the youngest patients (0-25 years old) with 22.9 vs 7.9 and 5.49% for patients aged (25-45) years and (45-65) years (p= 0.001, Table 2).

DISCUSSION

The primary endpoint of treatment efficacy was the proportion of patients with undetectable plasma viral load (CV<50 copies mL^{-1}) by treatment duration. Virological success was defined by a plasma viral load below the detection limit of the test used (CV<50 copies mL^{-1}), the virological evolution as being a reduction in the level of plasma CV (CV<1000 copies mL^{-1}) but not resulting in an undetectable viral load. Virological failure means either a viral load rebound (CV>1000 copies mL^{-1} after having been previously undetectable), or a viral load>1000 copies mL^{-1} following two successive measurements after 18 months of treatment.

Assessing the effectiveness of ARV treatment remains important in the response to HIV infection because it helps to know the best triple therapy regimen to give to patients. It makes it possible to make recommendations for programs to combat HIV/AIDS.

Patients were predominantly female, with a frequency of 71.2% in our cohort. Other studies have shown similar results such as those obtained in Lesotho by Labhardt *et al.*[16], who found a prevalence of 66.4%. This feminization of HIV infection is explained on the one hand by the fact that women are more vulnerable than men, because they are anatomically more susceptible to contracting infections. Studies done in Zimbabwe also indicate that the following factors such as inadequate support infrastructure for women living with HIV/AIDS, women's poverty, the unfair distribution of roles between the sexes, segregation and differentiation are responsible for the phenomenon of feminization of HIV infection[17]. The median age was 33 years old. As 39.8% of patients were between 25 and 45 years old and represented the majority of the study population. In Cameroon the average age was 39, with extremes of 17 and 88 years[18]. These results showed that the sexually active adult population was more affected by HIV infection. This may be due, on the one hand, to the fact that 90% of HIV infections in children result from mother-to-child transmission, that is to say during pregnancy, breast feeding or childbirth[19]. Effective treatment with an undetectable viral load has become an effective way of preventing mother-to-child and sexual transmission. There are three key categories for HIV viral load measurements: Unsuppressed (>1000 copies mL^{-1}), suppressed (detected but≤1000 copies mL^{-1}) and undetectable (viral load not detected by test used) according to the latest WHO report of July 2023.

We did not note any patients requiring 18 months of treatment to achieve undetectable status. However, those who were undetectable at 6 and 12 months remained undetectable, i.e., 80.2%. As 86.9% have had their viral load suppressed (<1000 copies mL^{-1}).

Studies done in Lesotho by Labhardt *et al.*[16] showed similar results in 86.7% of patients with virologic suppression. A positive virological evolution was noted in 6.7% of patients showing a good reduction in the rate of CV. At 6 months, 6.7% of patients had an undetectable plasma viral load. At 12 months, 73.5% of patients had an undetectable viral load,

virological suppression was greater, suggesting that most patients need a period of one year to have virological success. This result is comparable with that of Gallant *et al.*[20] which was that 70% of patients had an undetectable viral load after 1 year on a similar regimen, AZT+3TC+EFV. A Ugandan study with an AZT+3TC+EFV regimen showed an undetectability rate of 71.4% after 7.5 months, i.e., an earlier achievement of virological suppression[21]. This can be explained by greater *in vitro* efficacy on wild strains and resistant HIV-1 variants[22] and therapeutic failure was lower with EFV compared with NVP[23,24].

Virological failure was noted in 13% of patients in our cohort and this could be due to poor compliance or HIV-1 resistance mutations. A similar failure rate of 10% was found in a study in California after 12 weeks of ART AZT+3TC+NVP[25].

Gender had a significant influence on the success of the treatment of patients mainly at 12 months, viral suppression was significantly greater in women with 80.5% against 56.3% for men, ($p = 0.001$). The virological failure rate (CV>1000 copies mL^{-1}) was higher in men (20.3%) compared to 10% for women after 18 months of ART ($p = 0.001$). There is no data explaining this gender difference. In ARV therapeutic care, the better results in the female sex, suggest a hypothesis that women may have better compliance with treatment. Age was related to virological suppression and it was higher in the elderly than in young patients ($p = 0.001$) and conversely, treatment failure was higher in young people.

The limitations of this study were the lack of information on adherence, observation and side effects of treatment. Antiretroviral potency, immunological reconstruction, tolerability and drug-related toxicity are important factors to include in evaluating the efficacy of antiretroviral therapy. The patient information file did not include information on treatment compliance, adverse drug effects and ARV resistance, so the link between virological failure, compliance and resistance to treatment could not be studied.

SIGNIFICANCE STATEMENT

The study was to evaluate the efficacy of the emergence of several types of ARV gives rise to the possibility of several treatment combinations. This type of study provides guidelines for national HIV programs. Results showed that despite the appearance of new ARVs, the AZT+3TC+NVP regimen remains effective and less expensive, especially in countries with limited resources. therapeutic regimen in the treatment of HIV-1 infection. At 12 months, patients aged had significantly greater viral suppression than the youngest ($p = 0.001$). At the end of 18 months, the majority of patients were virally suppressed and senior patients had a better virological response to treatment.

CONCLUSION

Results had shown the efficacy of the AZT+3TC+NVP regimen in viral suppression after a treatment period of at least 1 year in HIV-1 patients. Viral suppression was related to patient gender and age. The ideal duration to obtain a good rate of undetectability is at least 12 months of treatment. Despite the desire of several countries to revise the ARV therapeutic lines for HIV, the AZT+3TC+NVP regimen remains effective and inexpensive. Monitoring of the appearance of HIV resistance by genotyping is necessary for better use of these first-line ART types.

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