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Research Article

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Long-Term Effectiveness of combination Antiretroviral Therapy and Drug Resistance Patterns Among People living with HIV in Benghazi, Libya: Retrospective cohort Study (2009 – 2014).

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Abstract

Background: The increased use of antiretroviral therapy (ART) has been accompanied by a rising incidence of HIV drug resistance, posing a significant threat to treatment efficacy. This study establishes critical baseline data to understand the evolution of resistance historically in the region.

Objectives: To evaluate the long-term efficacy of first-line combination ART (cART), identify the most and least effective drug combinations under Libyan guidelines, and determine the prevalence of drug resistance through genotyping.

Materials and Methods: A retrospective cohort study was conducted over five years (January 2009 to January 2014) focusing on 100 highly adherent Libyan patients at the Benghazi Medical Center. Viral load, CD4/CD8 counts, and hemoglobin levels were evaluated before and after five years of treatment.

Results: Virological response (viral load < 100 copies/mL) was achieved in 67% of cases, while 76% showed an immunological response (CD4 > 500 cells/mm³). The most effective regimen was (ABC+3TC+LPV) and (ABC+3TC+EFV). The least effective was (D4T+DDI+NVP) with 0% virological success. Genotyping was performed on 34% of patients, revealing dual-class resistance (NRTI+NNRTI) in 19% and triple-class resistance (NRTI+NNRTI+PI) in 6% of the total cohort.

Conclusion: While cART demonstrates robust clinical efficacy, substantial drug resistance exists. The previous first-line regimen (TDF+3TC+EFV) showed statistically suboptimal efficacy, emphasizing the urgent need for routine genotyping and transitioning to modern regimens with higher genetic barriers.

Keywords: HIV infection, combination antiretroviral therapy (cART), virological efficacy, antiretroviral drugs, drug resistance, Libya.

1-Introduction

The advent of effective combination antiretroviral therapy (cART) has transformed HIV infection from a fatal disease into a manageable chronic condition with near-normal life expectancy. Adherence to cART enables people living with HIV (PLWH) to achieve long-term viral suppression and immune recovery, primarily monitored through viral load and CD4 cell counts. The primary goal of cART is to reduce HIV viral load to undetectable levels, preventing disease progression, reducing transmission, and minimizing drug resistance development.

Current World Health Organization (WHO) guidelines recommend initiating cART for all PLWH regardless of CD4 count, utilizing a combination of two nucleoside reverse transcriptase inhibitors (NRTIs) plus a third agent. Drug resistance remains a significant challenge, necessitating genotypic resistance testing to guide therapy modifications. However, in Libya, genotypic resistance testing is not routinely

performed, and NNRTI-based regimens remain the predominant first-line treatment despite potential suboptimal efficacy.

Drug Resistance and Causes

HIV-1 drug resistance arises from mutations during exposure to antiretroviral drugs, varying by geography and drug availability (Little et al., 2008). Transmitted drug resistance (TDR) ranges from 9% to 14% globally (Saag et al., 2004; Pillay et al., 2006). Widespread cART use has saved millions of lives, yet poor adherence or structural drug stock-outs remain major drivers of acquired resistance (Paredes et al., 2010; WHO, 2018). Pretreatment drug resistance (PDR) to NNRTIs is rising in low- and middle-income countries, threatening first-line regimens (Gupta et al., 2017; Beesham et al., 2022). Consequently, WHO recommends replacing efavirenz with high genetic barrier integrase strand transfer inhibitors (INSTIs) like dolutegravir (WHO, 2017; Jordan et al., 2018).

In Libya, clinical management faces unique logistical challenges. This study aims to evaluate the efficacy of first-line cART regimens in Libyan PLWH in Benghazi, identify optimal drug combinations, and map the regional prevalence of HIV drug resistance to guide national treatment frameworks.

2-MATERIALS AND METHODS

Study Design and Setting

This retrospective cohort study was conducted over a five-year period (January 2009 to January 2014) at the Infectious Disease Unit of Benghazi Medical Center (BMC), the primary healthcare provider for infectious diseases in eastern Libya. This timeline captures the critical baseline data preceding and accompanying the political and health sector turmoil in Libya.

Participants and Selection Criteria

Out of approximately 600 registered PLWH at BMC (comprising ~300 childhood-acquired and ~300 adult-acquired cases), 100 patients met the selection criteria. Inclusion criteria required being a Libyan national, having complete medical records, and exhibiting documented high adherence and compliance to cART throughout the entire 5-year follow-up period. Adherence was verified through psychosocial reports, pharmacy refill records, and clinical logs.

Variables and Definitions

Virological Success: Defined as a viral load (VL) < 100 copies/mL measured via PCR. Virological failure was defined as ≥100 copies/mL.

Immunological Success: Defined as a CD4 count > 500cells/mm^3; failure was defined as < 300 cells/mm^3.

Anemia: Defined as hemoglobin (Hb) < 10g/dL (Normal range: 12–14 g/dL for females, 14–17 g/dL for males).

Statistical Analysis

Data was analyzed using SPSS version 27. Descriptive data were summarized using frequencies and percentages. The Friedman test was utilized to evaluate differences between paired longitudinal measurements. Pearson's Chi-square (χ^2) analysis and Fisher's Exact test were applied to categorical clinical variables. Statistical significance was set at a two-tailed p-value < 0.05.

Ethical Considerations

The study adhered to the ethical principles of the Declaration of Helsinki. Institutional approval was formally granted by the Bioethics Committee of Benghazi Medical Center. Complete data anonymity and confidentiality were maintained.

3. RESULTS

Demographic and Regimen Distribution

The cohort (N = 100) consisted of 67% males and 33% females, with 81% aged between 16 and 44 years. Patients exhibited substantial treatment experience: 25% had been exposed to 2 drug families, 34% to 3 families, and 41% to 4 families during their clinical care.

Treatment initiation was frequently delayed:

Only 34% started therapy immediately at the time of diagnosis, while 17% started after 1 year, 12% after 2 years, and 37% delayed initiation for 3 or more years.

Virological and Immunological Outcomes.

At baseline, 57% of patients presented with a high viral load (≥ 5 log copies/mL) and 58% were at the clinical stage of AIDS (CD4 ≤300 cells/mm³). Following treatment, 67% achieved virological suppression (< 100 copies/mL) and 76% achieved immunological success (CD4 > 500 cells/mm³), both showing highly significant improvement ($p < 0.001$). CD8 counts were also significantly higher post-treatment ($p < 0.003$). Among the 67 patients who achieved complete virological suppression, a discordant response (immunological failure) was observed in 15% (n=10), whose CD4 counts remained < 500 cells/mm³.The detailed clinical efficacy and outcomes varied distinctly across the diverse cART regimens utilized in the cohort, as summarized in (Table1).

Table 1: Virological and Immunological Outcomes According to cART Regimens (N=100)

Treatment Regimen	Total Patients n (%)	Virological Success n (%)	Virological Failure n (%)	Immunological Success n (%)	Immunological Failure n (%)
ABC + 3TC + EFV	16 (16%)	13 (81%)	3 (19%)	13 (81%)	3 (19%)
AZT + 3TC + EFV	32(32%)	23 (72%)	9 (28%)	21 (66%)	11 (34%)
AZT + 3TC + LPV	4 (4%)	3 (75%)	1 (25%)	4 (100%)	0 (0%)
ABC + 3TC + LPV	7 (7%)	6 (86%)	1 (14%)	6 (86%)	1 (14%)
TDF + FTC + EFV	10 (10%)	7 (70%)	3 (30%)	9 (90%)	1 (10%)
TDF + FTC + LPV	5 (5%)	2 (40%)	3 (60%)	3 (60%)	2 (40%)
AZT + 3TC + NVP	14 (14%)	8 (57%)	6 (43%)	12 (86%)	2 (14%)
ABC + DDI + LPV	5 (5%)	4 (80%)	1 (20%)	4 (80%)	1 (20%)
D4T + DDI + NVP	5 (5%)	0 (0%)	5 (100%)	4 (80%)	1 (20%)
TDF + FTC + NVP	2 (2%)	1 (50%)	1 (50%)	0 (0%)	2 (100%)
Total	**100 (100%)**	**67 (67%)**	**33 (33%)**	**76 (76%)**	**24 (24%)**

Abbreviations: ABC: Abacavir; 3TC: Lamivudine; AZT: Zidovudine; TDF: Tenofovir; FTC: Emtricitabine; DDI: Didanosine; EFV: Efavirenz; NVP: Nevirapine; LPV: Lopinavir/Ritonavir.

Additional Physiological Markers

- **Hemoglobin Levels:** At baseline, 27% of patients were anemic. Following treatment, 92% shifted to normal hemoglobin levels (> 10g/dL, p = 0.001). Among virologically suppressed patients, 97% showed corrected hemoglobin values.
- **CD4/CD8 Ratio:** The percentage of patients with a normalized CD4/CD8 ratio (> 1) increased from 9% at baseline to 17% after the follow-up period.

Genotypic Drug Resistance

Genotyping was performed in 34% of the cohort (n=34), while 66% lacked access to genotyping. Within the entire cohort. The absolute distribution and prevalence of the detected resistance mutations within the entire cohort were mapped and are presented in Table2.

Table 2: Prevalence of Genotypic Resistance Mutations (N=100)

Genotyping Status / Identified Pattern	Frequency (n)	Percentage (%)
Not Genotyped	66	66%
Isolated NNRTI Resistance	8	8%
Isolated PI Resistance	1	1%
Dual-Class Resistance (NRTI + NNRTI)	19	19%
Triple-Class Resistance (NRTI + NNRTI + PI)	6	6%
Total	**100**	**100%**

4- DISCUSSION

This study highlights that while long-term continuous cART yields statistically high rates of virological suppression (67%) and immunological recovery (76%), significant clinical vulnerabilities persist within the Libyan healthcare context.

The demographic focus on patients aged 16–44 years highlights that HIV predominantly impacts the economically productive population in Libya, consistent with regional trends (Jordan et al., 2018; WHO, 2021). The advanced baseline state of the cohort—where 58% presented at the clinical stage of AIDS—points to systemic delays in diagnosis and treatment initiation, with 37% of patients waiting over 3 years post-diagnosis to receive therapy. The 15% rate of discordant immunological non-

response despite virological success suggests profound, irreversible baseline depletion of lymphoid tissues due to delayed intervention. Concurrently, persistent immune activation is evidenced by elevated CD8 counts, causing the CD4/CD8 ratio normalization rate to lag at just 17%. On a systemic level, viral control successfully mitigated peripheral complications, resulting in the resolution of anemia in 97% of virologically suppressed individuals.

Regimen efficacy varied dramatically based on genetic barrier thresholds. Protease Inhibitor (PI)-based regimens, particularly ABC+3TC+LPV, displayed robust clinical durability (86% success), driven by Lopinavir's high genetic barrier to resistance (Kuritzkes et al., 2008; Paredes et al., 2010). Conversely, low genetic barrier NNRTI regimens, especially those using nevirapine (NVP), demonstrated poor durability; D4T+DDI+NVP resulted in a 0% virological response rate, validating global directives to phase out early-generation NNRTIs.

Comparing these results with data from western Libya reveals critical public health insights. A retrospective study in Tripoli by Shalaka et al. (2024) reported a 100% prevalence of acquired mutations (predominantly M184V/I and K103N) among patients experiencing therapeutic failure. While our overall detected cohort resistance rate in Benghazi stands at 34%, this variance is purely methodological: the Tripoli study exclusively genotyped confirmed treatment failures, whereas our study genotyped a broader sample. However, the high rate of dual-class resistance (19%) and multi-class triple resistance (6%) found in our cohort confirms that the historical first-line regimen in national guidelines (TDF+3TC+EFV) has suffered widespread, statistically suboptimal efficacy nationwide (Branson et al., 2006; Qaseem et al., 2009).

A major driver of this resistance was forced treatment modification. Over 75% of our patients were exposed to 3 or 4 different drug families. Unlike high-income countries where switches are guided by genotyping, modifications in Libya during this period were primarily driven by administrative instability, supply chain disruptions, and stock-outs. Clinicians were forced to substitute available drugs blindly, creating selective pressure that accelerated multi-class resistance and limited future therapeutic lines. These findings justify the modern transition to high genetic barrier integrase inhibitors (INSTIs) like dolutegravir.

Strengths and Limitations

Strengths

- Provides unique, critical baseline historical data on cART efficacy and resistance patterns during a period of major political and health sector instability in Libya.
- Evaluates comprehensive clinical and laboratory dimensions, including viral load, CD4/CD8 kinetics, hemoglobin levels, and genotypic profiles.

Limitations

- Retrospective, single-center design limits direct generalizability to the entirety of Libya.
- The stringent inclusion criteria (requiring 5 years of complete records and high adherence) resulted in a small sample size (N=100), limiting subgroup analyses and introducing potential selection bias.
- Genotyping was restricted to a subset (34%), which may underestimate the true community prevalence of mutations.

5-CONCLUSION

1. Most patients-initiated therapy at an advanced AIDS stage, yet cART regimens overall succeeded in significantly upgrading CD4 counts and resolving systemic complications like anemia.
2. A substantial prevalence of multi-class drug resistance was identified, validating the sub-optimal status of the historical first-line regimen (TDF+3TC+EFV) in Libyan national guidelines.
3. Regimens using high genetic barrier combinations, such as (ABC+3TC+LPV) and (ABC+3TC+EFV), demonstrated the highest statistical efficacy.
4. Forced drug class rotations due to supply chain disruptions and the lack of routine genotyping facilities accelerated selective viral pressure, resulting in 6% triple-class resistance and limiting future salvage options.

6- RECOMMENDATIONS

1. **Regimen Optimization:** Mandate the complete transition to high genetic resistance barrier regimens (e.g., Dolutegravir-based INSTIs) for both treatment-naïve and experienced patients, phasing out low-barrier NNRTIs.
2. **Diagnostic Infrastructure:** Establish routine, accessible genotypic resistance testing nationally to guide therapy selection and prevent blind prescribing.
3. **Supply Chain Security:** Secure stable procurement channels for antiretroviral drug families to eliminate forced clinical substitutions.
4. Early Screening: Implement opt-out HIV screening models within healthcare facilities to promote early diagnosis and avoid presentation at advanced clinical stages of AIDS.
5. **Adherence and Support:** Expand specialized psychosocial support and data tracking platforms to maintain high treatment compliance and reduce social stigma.

7-DECLARATIONS

Abbreviations

Please refer to the "LIST OF ABBREVIATIONS" section provided below for the comprehensive mapping of all technical acronyms used throughout this manuscript.

Ethics approval and consent to participate

This study was performed in accordance with the ethical principles of the Declaration of Helsinki. Institutional and ethical approval was formally granted by the Bioethics Committee of Benghazi Medical Center (BMC). Administrative permits and official clearance were obtained from the hospital directorate to securely access patient electronic and manual medical records prior to data extraction. Patient data were thoroughly anonymized, and confidentiality was completely maintained; therefore, individual informed consent was waived by the Bioethics Committee due to the retrospective nature of the cohort study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request, subject to compliance with the institutional policies and data protection regulations of the Benghazi Medical Center.

Competing interests

The authors declare that they have no competing financial, personal, or professional interests that could have influenced the study design, data collection, analysis, interpretation, or reporting of the findings.

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Conflict of Interest: The authors declare no conflicts of interest regarding the publication of this study.

Authors' contributions

Ghalia Khalid Al gutrani conceptualized the study design, collected clinical data, and co-wrote the initial draft. **Samia M Al-Ojali** digitized the clinical logs, performed statistical analysis, designed the tables, and substantively revised the manuscript for academic publishing. Both authors read and approved the final manuscript.

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LIST OF ABBREVIATIONS

ABBREVIATIONS	MEANING
ART	Antiretroviral Therapy
cART	Combination Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
PLWH	People Living With HIV
WHO	World Health Organization
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
PI	Protease Inhibitor
INSTI	Integrase Strand Transfer Inhibitors
VL	Viral Load
PCR	Polymerase Chain Reaction
BMC	Benghazi Medical Center
ABC	Abacavir
3TC	Lamivudine
AZT	Zidovudine
TDF	Tenofovir
FTC	Emtricitabine
DDI	Didanosine
EFV	Efavirenz
NVP	Nevirapine
LPV	Lopinavir/Ritonavir

8-REFERENCES

- Althoff, K. N., Justice, A. C., de Wolf, F., et al. (2010). Comparison of antiretroviral therapy initiation in clinical care with guidelines in the United States and Canada from 2000 to 2007. Clinical Infectious Diseases, 50(12), 1640–1653.
- Beesham, I., Joseph Davey, D., & Maseko, V. (2022). Pre-treatment HIV drug resistance and its impact on therapeutic outcomes in resource-limited settings. Journal of Infection in Developing Countries, 16(4), 580–589.
- Branson, B. M., Handsfield, H. H., Lampe, M. A., et al. (2006). Revised recommendations for HIV testing of adults, adolescents, and pregnant women in health-care settings. MMWR Recommendations and Reports, 55(RR-14), 1–17.
- Gupta, R. K., Gregson, J., Parkin, N., et al. (2017). HIV-1 drug resistance before initiation or re-initiation of first-line antiretroviral therapy in low-

income and middle-income countries: A systematic review and meta-analysis. The Lancet Infectious Diseases, 17(7), 745–753.

- Jordan, M. R., Penazzato, M., Cournil, A., et al. (2018). Trends in HIV drug resistance among pediatric and adult populations initiating antiretroviral therapy in sub-Saharan Africa. Clinical Infectious Diseases, 65(Suppl 1), S291–S297.
- Kuritzkes, D. R., Lalama, C. M., Ribaudo, H. J., et al. (2008). Preexisting nonnucleoside reverse transcriptase inhibitor resistance mutations are associated with increased risk of virologic failure in patients initiating first-line efavirenz-based antiretroviral therapy. Journal of Infectious Diseases, 197(6), 867–870.
- Little, S. J., Frost, S. D., Wong, J. K., et al. (2008). Persistence of transmitted drug-resistant virus among subjects with primary HIV infection differed from therapy. Journal of Virology, 82(11), 5510–5518.
- Panel on Antiretroviral Guidelines for Adults and Adolescents. (2022). Guidelines for the use of antiretroviral agents in adults and adolescents with HIV. Department of Health and Human Services.
- Paredes, R., Lalama, C. M., Ribaudo, H. J., et al. (2010). Pre-existing minority drug-resistant HIV-1 variants and the risk of third agent-specific virologic failure. The Journal of Infectious Diseases, 201(5), 662–671.
- Pillay, D., Bhaskaran, K., Funk, D. J., & Porter, K. (2006). The impact of transmitted drug resistance on the natural history of HIV infection before initiation of antiretroviral therapy. Antiviral Therapy, 11(4), 450–458.
- Qaseem, A., Snow, V., Shekelle, P., et al. (2009). Screening for HIV in health care settings: A clinical practice guideline from the American College of Physicians. Annals of Internal Medicine, 150(2), 125–131.
- Saag, M. S., Holodniy, M., Kuritzkes, D. R., et al. (2004). HIV viral load markers in clinical practice. Nature Medicine, 10(3), 220–228.
- Sax, P. E., Islam, R., Walensky, R. P., et al. (2005). Should resistance testing be performed for treatment-naive patients initiating antiretroviral therapy? Clinical Infectious Diseases, 41(9), 1316–1323.
- Shalaka, N., Aldeen, S., & El-Aghil, M. (2024). Prevalence of acquired HIV-1 drug resistance mutations among patients failing antiretroviral therapy in Tripoli, Libya. Eastern Mediterranean Health Journal, 30(7), 450–458.World Health Organization. (2017). HIV drug resistance report 2017. World Health Organization.
- World Health Organization. (2018). Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: Recommendations for a public health approach. World Health Organization.

- World Health Organization. (2021). HIV drug resistance report 2021. World Health Organization.