



Research article

Immuno-virological status and its associated factors among HIV-positive patients receiving highly active antiretroviral therapy at delgi primary hospital, northwest Ethiopia, 2020/2021: A cross-sectional study

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ABSTRACT

Background: Highly active antiretroviral therapy (HAART) improves clinical outcomes by suppressing viral replication and allowing immune reconstitution. It also reduces HIV-related complications including morbidity, mortality, and extended hospitalizations for HIV-positive individuals. Regular assessment for antiretroviral treatment response is fundamentally important to address the factors associated with the poor clinical outcome including immunologic failures among HIV-positive patients on HAART. Therefore, this study aimed to investigate the immuno-virological status and describe its determinants among HIV-positive patients receiving HAART at Delgi primary hospital, Northwest Ethiopia.

Methods: A hospital-based cross-sectional study was conducted at Delgi primary hospital from October 25th through June 19th 2021 among a total of 442 study participants. A systematic random sampling technique was employed to enrol participants in the study. Socio-demographic and clinically related data were collected using a semi-structured questionnaire. About 3–5 ml of venous blood was collected aseptically for CD4⁺ T cell count and viral load test. SPSS version 20 software was used for statistical analysis. Bivariate and multivariate logistic regression analyses were conducted to determine the factors associated with immuno-virologic status among HIV-positive patients on HAART. The odds ratio with 95% CI was computed to determine the strength of association. Then, a p-value < 0.05 was considered a statistically significant association. For this study, the results were presented by using frequency summary tables, and texts.

Results: Among the total study participants, 283 (64%) were males and the mean age of the study participants was 37 ± 11.5. The overall immunological and virological failure among highly active antiretroviral therapy (HAART) receiving participants was found to be 9.5% (42/442, 95%CI:3.23–15.09) and 12.2% (54/442, 95% CI: 2.81–23.04) respectively. In the multivariate analysis, study participants with age ≥50 years old [AOR = 1.97, p = 0.01, 95%CI (0.02–4.03)], participants having current viral load count greater ≥1000 copies/ml [AOR = 3.97, p = 0.03, 95%CI (1.09–5.01)] and having TB-co-infection [AOR = 2.51, p = 0.05, 95%CI (1.02–7.51)] were statistically associated with increased risk of immunological failure. Similarly, TB-coinfected participants were 1.88 (95%CI = 0.89–10.02) times at greater risk for virological failure.

Conclusion: In this study, the magnitude of immuno-virological failure is alarming. This may be shown the need for integrated and substantial commitment to enhancing patient antiretroviral treatment adherence in the study area. Also, regular assessment for antiretroviral treatment response is fundamentally important to address the determinants associated with virological and immunologic failures among HIV-positive patients taking HAART. Furthermore, early initiation of HAART may be imperative to achieve favourable virological suppression and immunological reconstitution.

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1. Introduction

Acquired immunodeficiency syndrome (AIDS) caused by the human immunodeficiency virus (HIV) is a viral infection that attacks the body's immune system, specifically the white blood cells called CD4⁺ T cells [1]. This can be made individuals more susceptible to several infections including bacterial, viral, and fungal agents [2, 3]. According to the United Nations for HIV/AIDS program also known as "UNAIDS" 2019 data report [4], an estimated 37.9 million people (36.2 million adults and 1.7 million children) are living with HIV and only 68% of adults and 53% of children are receiving lifelong antiretroviral therapy (ART) but, 12.6 million people are still waiting for treatment worldwide.

Sub-Saharan Africa has remained among the hardest hit regions provided that one in every 25 adults is living with HIV/AIDS. The region accounts for nearly two-thirds of the global total HIV cases [5]. In Ethiopia, HIV has remained a major public health threat and top public health concern despite the integrated implementation of ART since 2005 [6]. According to the report of the Ethiopian federal ministry of health on HIV response [7], out of a total of 593,400 people living with HIV/AIDS (PLWHA), only 298,512 people were accessible to highly active antiretroviral therapy (HAART). Similarly, based on the global AIDS update 2020, a total of 670,000 Ethiopian people of all age groups are living with HIV/AIDS [8]. However, the annual number of PLWHA has shown declining trends over the past couple of decades in Ethiopia. The prevalence decreased from 3.3% to 0.9% from 2000 to 2017 with declining of 83 000 to 15 600 AIDS-related deaths in the year 2000–2017 [5,7,9].

Increasing medical access to effectively contain HIV transmission, advancing laboratory diagnosis, therapeutics and early prevention and control of many opportunistic infections (OIs) have enabled PLWHA to live long and improve their healthy lifestyles [2, 6]. Several OIs like pulmonary tuberculosis (TB), bacterial pneumonia, cryptococcal meningitis, Herpes zoster, oral candidiasis, and fungal pneumonia remain the leading cause of death among HIV-positive persons [3] given that TB account for around one in three AIDS-related deaths globally [8].

HAART is a lifesaving treatment for HIV-positive individuals by increasing viral suppression, improving their quality of life, improving immune reconstitution, and reducing HIV-related complications including morbidity, mortality rates, and extended hospitalizations that expose to severe nosocomial infections [10, 11]. HIV infection transmission can be high due to virological, immunological, and clinical failure [12]. Evidence has shown that virological, clinical, and immunological treatment failures are the most experienced events among HIV-positive individuals. Among these, virological failure is a common phenomenon at HIV-positive persons encountered after starting treatment [10, 13].

Based on the World Health Organization (WHO) guidelines for ART, virological failure is conceded when the plasma viral load exceeds 1000 copies/ml persistently at least 6 months after starting a new ART regimen with adherence support. Also, immunological failure is confirmed when CD4⁺ cells count falls to the baseline or persistent CD4⁺ levels below 100 cells/mm³ [14]. Treatment failures among HIV infected patients are usually accompanied by poorer clinical outcomes than the responses to ART [15]. HIV-infected patients on HAART have shown a remarkable reduction in AIDS-related clinical impacts such as mortality, morbidity, and associated psychosocial problems. This is because the risk to develop AIDS-related mortality and the poorer therapeutic outcome are heavily dependent on the immune system, viral suppression, and the supportive nutritional status of the individuals [16, 17, 18].

Results from observational studies have revealed that at least 73.5% of HIV-positive individuals have shown complete virological suppression within 6 months [19]. This can be explained as immuno-virological discordance which is mainly associated with the CD4⁺ cell count nadir before initiation of ART and the increased risk of progression to death [13, 20, 21].

Despite no curative therapy for HIV currently, the introduction of HAART has shown a substantial decrease in HIV related problems with improved patient survival and quality of life. However, HIV-positive patients receiving HAART are suffering from several major challenges such as poor adherence to medications (adherence to prescribed ART), defects in host immunity, the emergence of HIV-drug resistance due to viral replication capacity and pre-existing polymorphisms, drug-related problems (toxicity, drug potency, drug-drug interactions) and many other socio-economic conditions, particularly among many developing nations [10, 14, 22]. The uses of HAART assist in improving the long-term virological result as well as the clinico-immunological success by decreasing the plasma viral load to an undetectable level [23]. Moreover, the virological assessment offers an early prognostic indicator of therapeutic outcome, therapeutic switching (from first-line to second-line), reduces drug resistance, and improves clinical outcomes over clinical and immunological monitoring [14]. However, due to the high costs, trained manpower, test biosafety requirements, and available infrastructures, clinical and immunological monitoring are usually advised to start and monitor the efficacy of HIV therapy [10, 13, 16, 20].

Risk factors associated with immunological, virological, and treatment failure may include several Socio-demographic variables, baseline data, and clinical characteristics [24, 25, 26]. As a result, a clear understanding of risk factors that are associated with poor clinical outcomes (immunological, virological, and treatment failure) could help in addressing the risk of poor therapeutic outcomes. To the investigators' knowledge, no study was conducted to assess the immune-virological status and factors contributing to the poor treatment outcome among HAART receiving patients in our study area. Therefore, this study aimed to investigate the immunological and virological outcome of HAART receiving HIV-positive individuals attending the ART center of Delgi primary hospital.

2. Materials and methods

2.1. Study design, setting, and period

A hospital-based cross-sectional study was conducted from October 25th 2020 to June 19th 2021 to investigate the immuno-virological status and associated determinants among HIV-positive people on HAART at Delgi primary hospital, Northwest Ethiopia. The hospital is found in Delgi town of central Gondar Zone of the Amhara Region, Northwest Ethiopia. It is 800 km away from Addis Ababa which is the capital city of Ethiopia. The town has a latitude and longitude of 11051'N 380 1'E with an elevation of 2706 m above sea level. Based on the estimates from the Central Statistical Agency of Ethiopia conducted in the 2011 Ethiopian calendar, the town has an estimated total population of 1,214,172. Delgi primary hospital can hold more than 100 beds, has 175 health care providers, and has 80 administrative and technical staff. Generally, the hospital is delivering a service for a population of about 1 million with an annual average client flow of 350,125 people. Also, the hospital is providing a service for a large number of people coming from the surrounding zones and nearby regions for both out-patient and in-patient services. The hospital was officially launched in 2013 and currently provides an ART centre for more than 1500 HIV-positive patients of whom 650 were HAART receiving patients.

3. Source population and study population

The source population for this study was all HIV-positive individuals who were attending the ART center for their regular follow-up during the study period. Similarly, all HIV-positive adult persons (age ≥18 years old) who were receiving HAART for at least 6 months were used as study participants to assess the immune-virological and associated determinants.

4. Eligibility criteria

4.1. Inclusion criteria

The study participants who were included in this study were: HIV-positive patients with at least 6 months of follow-up on HAART, adults HIV positive individuals with age ≥ 18 years old who were voluntary and avail to participate with written informed consent during the survey, HIV-positive patients who were with complete socio-demographic information (age, gender, educational level, occupation, marital status, residence, and socioeconomic status), clinical data (duration on ART, any prophylaxis initiated, the status of opportunistic infections, functional status, therapeutic switching, level of ART adherence, body mass index and WHO clinical stage) as well as essential laboratory data upon chart review such as baseline CD4⁺ T cell count were included in the study.

4.2. Exclusion criteria

HIV-positive patients who refuse to take consent, those patients who were seriously sick, unconscious and those who had incomplete baseline socio-demographic data, clinical or laboratory records (CD4⁺ T cell count, ART adherence, drug regimen types, WHO clinical stage, body mass index, hemoglobin level and history of microbial co-infections or comorbidity) were excluded from the study. Besides, pregnant women, Coronavirus disease-19 (COVID-19) co-infected patients, those who had lost to follow-up, febrile, and transfer in/out cases were excluded.

5. Sample size determination and sampling technique

The required sample size for this study was determined based on the equation used for the estimation of a single population proportion ($n = [(Z \alpha/2)^2 P (1-P)/d^2]$) by taking the assumption of 50% (since there was no prior similar study in the study area) for population estimation prevalence ($p = 0.5$), using a 95% confidence interval (95%CI; $Z_{\alpha/2} = 1.96$) and the margin of error ($d = 0.05$). Then, the required sample size for the study by substituting the above values was 384.2. By adding a 15% non-response rate ($384 \times 0.15 = 57.6$), the total sample size required for the study was approximately 442 study participants. A systematic random sampling technique was employed to recruit the study participants attending the ART clinic during the study period. Based on the information from ART health care workers, an average of 15 HIV-positive patients per day were coming for regular ART follow-ups and gave blood for immunological and virological monitoring (i.e., CD4⁺ T cell count and viral load determination) concurrently. During the 8-months of the data collection period, 650 HIV-positive patients receiving HAART for at least 6 months were expected to visit the ART clinic for regular follow-up. The total number of PLWHA in our setting was 1500. The sampling interval (Kth value) for the study was computed using the formula, $K = 1500/442 = 3.00$. The first participant was selected simply by the lottery method. Then, every next study subject was selected in the order of every Kth interval of ART regular follow-up. Moreover, chart review and blood collection for immune-virological assessment were also made based on this scenario (Figure 1).

6. Study variables

6.1. Dependent variables

Immunological status (success or failure) and virological status (success or failure).

6.2. Independent variables

The independent variable includes socio-demographic variables (age, gender, residence, marital status, educational status, residence and inc-

ome level), clinical related information (treatment adherence, the presence/absence of microbial co-infection/opportunistic infections, functional status, types of ART drug, WHO clinical stage, and duration on ART), anthropometric measurements (body mass index) and laboratory data (baseline and current CD4⁺ T cell count, hemoglobin level well as HIV-1 plasma viral load).

7. Operational definition

Clinical failure: For adults and adolescents, clinical failure is conceded when new or recurrent clinical events indicating severe immunodeficiency at least after 6 months of ART initiation with supportive adherence [14].

Immunological failure: This is confirmed when CD4⁺ T cell count falls to the baseline or below or persistent CD4⁺ T cell count levels below 100 cells/mm³ [14].

Virological failure: Based on the 2016 WHO guideline, it is defined by a persistently detectable viral load exceeding 1000 copies/ml where two consecutive viral load measurements within a three-month interval based on adherence support between measurements after at least 6 months of starting a new ART regimen [14].

Baseline CD4⁺ count: The latest value measured before the initiation of HIV therapy [6, 14, 27].

HAART: It is the combination of antiretroviral treatment with at least three drugs, including at least one non-nucleoside reverse-transcriptase inhibitor (NNRTI) or a protease inhibitor (PI) and/or abacavir, or a treatment regimen with a combination of NNRTI and a boosted PI [6, 14, 27].

ART adherence: The status that was assessed based on the reported number of pills taken in the last one month divided by the number of prescribed pills and multiplied by 100%. It includes taking ART medications properly with no missing doses of the prescribed antiretroviral agents [10, 14, 25, 28].

$$\text{Adherence} = \frac{\text{No of the dose of ART taken}}{\text{No of prescribed doses of ART}} \times 100\%$$

Based on this, participants were categorized into groups.

- ✓ **Good adherence:** When the treatment adherence to ART of HIV-positive patients is greater than or equal to 95%.
- ✓ **Fair adherence:** When the treatment adherence by the patients ranges from 85% to 94%.
- ✓ **Poor adherence:** Treatment adherence by the patients is less than 85% or <85% doses.

8. Data collection tools

8.1. Socio-demographic and clinical-related data collection

Before the socio-demographic and clinical-related data collection, verbal and written participant information was provided to all study participants and their caregivers. Meanwhile, a semi-structured questionnaire to address the primary outcome of interest was adopted from various relevant kinds of literature to assess the socio-demographic variables of HIV-positive participants on HAART including age, gender, level of education, residence, marital status and others related to the study subject's clinical characteristics (duration of ART, adherence to ART, the history of co-infection/opportunistic infections, history of cotrimoxazole intake, functional status, WHO clinical stage, body mass index, baseline CD4⁺ T cell counts and types of ART drug) were extracted from the participant's medical record using their respective ART medical registration number. To maintain completeness, simplicity, and clarity of the questionnaire, 10% of the questionnaires were pre-tested at the University of Gondar referral hospital before the actual study was

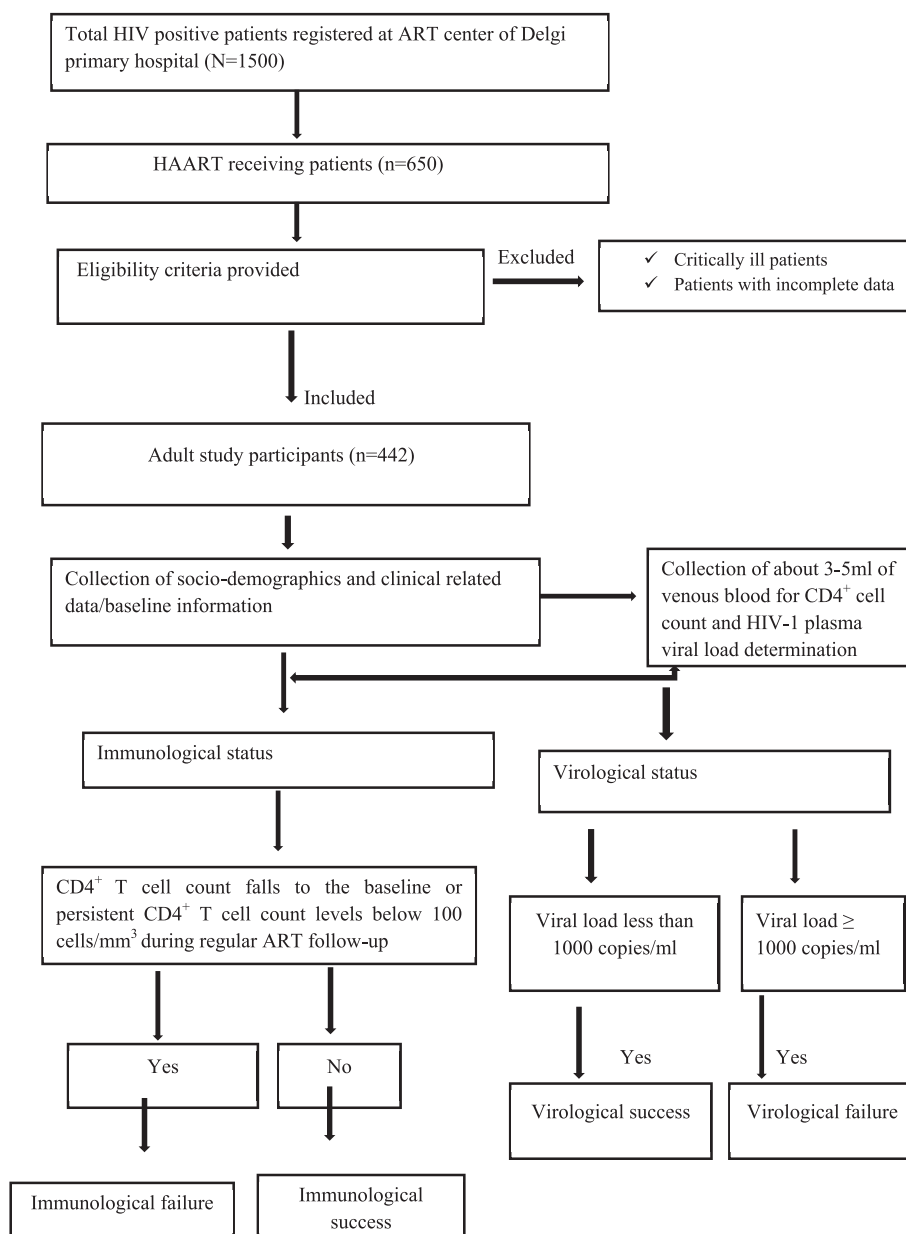


Figure 1. Schematic diagram depicting the overall workflow of the study protocol for HAART receiving HIV-positive participants at Delgi hospital in the study period (October 25th 2020 to June 19th 2021).

processed. Subsequently, minor revisions were made based on the respondents' test results.

9. Laboratory investigation procedure

9.1. Specimen collection, processing, transport, and handling

Following standard operational procedure (SOP) about 3–5 ml of venous blood was drawn from each study participant using a vacutainer tube separately in two tubes containing anticoagulant ethylene diamine tetra-acetic acid (EDTA) by trained laboratory personnel for CD4⁺ T cell count and viral load tests [6, 14, 29]. Because this study is cross-sectional, no triple regimen was used for the study participants. The laboratory procedure principally includes:

CD4⁺ T cell count: The specimen collection and testing were performed following standard clinical laboratory protocols and laboratory biosafety precautions. About, 3–5 ml of whole blood was collected from each study participant during regular follow-up visits following standard

blood collection procedures [30]. Then, each collected specimen was labelled with the participants' unique identification number and transported immediately to the ART laboratory for virological investigation [10, 31]. Thereafter, the specimen was subjected to centrifugation at 3000 rpm for 20 min and the plasma was separated and aliquots were prepared for further viral load testing. Specimens for CD4⁺ cell counts were stored at room temperature (20°C–25 °C) and processed within 24 h. Finally, quantification of absolute CD4⁺ T cell count from whole blood specimens was conducted using the BD FACS calibre flow cytometry system (BD, CA, USA) following both the manufacturer's protocol and the SOP designed particularly for laboratory work up in our set-up [30]. Adhering to the SOPs, the absolute CD4⁺ T cell count was conducted by adding 50 µL of whole blood to a reagent tube containing 20 µL of fluorochrome-labelled monoclonal antibodies in the reagent which can bind specifically to leucocytes surface antigens which were followed by mixing and incubation for 30 min at room temperature. Upon acquisition, the cells travel laser beam (488nm Argon) and scatter the laser light so that the stained cells generate fluoresce. Both the light scatters and

fluorescence signals provide sufficient information regarding the cells size, internal complexity and their relative fluorescence intensity [25, 29, 32, 33].

Plasma viral load determination: The aliquots that were prepared were transferred into a sterile screw-capped tube. The sample transportation for viral load determination has strictly adhered to the cold chain processes in line with the laboratory bio-safety precautions for pre-analytical, analytical, and post-analytical conditions of the laboratory work [10, 22]. The specimen transportation was made based on dry ice with triple packaging and stored at -80°C on its arrival at the University of Gondar referral hospital. Finally, the plasma viral load determination was conducted by using a quantitative real-time polymerase chain reaction HIV-1 assay [34] by the COBAS[®] AmpliPrep instrument (Roche, Homburg, Germany). The nucleic acid isolation from the HIV-1 positive participants during their regular ART follow-up was determined using COBAS[®] AmpliPrep instrument through an automated detection using the COBAS[®] TaqMan[®] analyzer. The test involves specimen preparation, reverse transcription of the target RNA to produce complementary deoxyribonucleic acid (cDNA), amplification of target cDNA as well as quantification of viral nucleic acid [16, 25, 27, 35]. The overall schematic flow chart for the study process is also summarized as shown in (Figure 1).

10. Data quality assurance

Quality control was established for each course of the study. The semi-structured questionnaire that was used for the collection of socio-demographic characteristics and clinical data was pre-tested before the actual data collection. Likewise, the questionnaire was first prepared in the English language from various literature and translated into the local language (Amharic version) and then transcribed back to English to maintain its consistency, simplicity, clarity, and completeness in light of the concept of the research question. Besides, adequate training was given for all data collectors and supervisors to ensure the quality of the study. As a result, filled questionnaires were double-checked for completeness and consistency daily by the principal investigator and the supervisors and kept confidentiality of the data. Meanwhile, quality control for laboratory investigation was kept by strictly following the SOPs during the pre-analytical (sample collection, handling, and transportation), analytical and post-analytical phases of the study. In each step of the study, all reagents used for testing were carefully checked for their expiry date and cross-checked that the reagents were prepared according to the manufacturer's instructions. Furthermore, quality control reagents during viral load determination (negative, low positive, and high-positive) and CD4+ count (low, medium, and high) were included in each test assay to evaluate the run validity.

11. Data processing and statistical analysis

We used SPSS version 20 software for data entry and analysis. Data were carefully entered, cleaned, and double-checked. Descriptive statistics were employed to compute the frequency, mean and standard deviations of continuous variables. Statistical data were summarized and organized using texts and frequency tables. Bi-variate and multivariate logistic regression analyses were conducted to measure the association between dependent and independent variables. In the bivariate logistic regression model, study variables with a p -value ≤ 0.2 were imported into the multivariate logistic regression model to control the cofounder effect so that its adjusted odds ratio (AOR) and 95% confidence intervals were recorded. Also, in the multivariate analysis, multicollinearity was considered by performing variance inflation factor to indicate the correlation of independent variables to each other. Moreover, Hosmer-Lemeshow goodness of fit statistic was considered at a p -value >0.05 for the logistic regression. A p -value of less than 0.05 was considered to determine the statistically significant association.

12. Ethical clearance

The study was approved by the Debre Tabor University, research, and ethical review committee. The research and ethical review committee permission letter's reference number was chs/224/2020. The permission letter signed by the concerned body was obtained and sent to Delgi primary hospital and other concerned bodies to be secured the study at all levels. After permission was obtained from the hospital administration, the potential benefits and the risks associated with participation in the study have been briefed to study participants. Consequently, written informed consent or assent was obtained from study participants and caregivers. All results were kept confidential and the process was solely made through coding rather than naming to ensure participants' privacy concerns. Finally, study participants who had shown the virological and immunological failures were linked to responsible health officials to receive proper interventions.

13. Results

13.1. Socio-demographic characteristics of the study participants

In this study, a total of 442 HIV-positive individuals on HAART were included with an overall response rate of 100% (442/442). Among the total study participants, 283 (64%) were males. The current age distribution of the study participants has shown that 213 (48.2%) were in the age range of 40–49 years followed by the age group of 18–24 years

Table 1. Socio-demographic characteristics of the study participants (n = 442) who were taking HAART at Delgi primary hospital, Northwest Ethiopia (October 25th 2020 to June 19th 2021).

Variable	Category	Frequency	Percentage (%)
Current age (years)	18–24	95	21.5
	25–39	51	11.5
	40–49	213	48.2
	≥ 50	83	18.8
Age at initiation of ART	18–24	115	26
	25–39	78	17.6
	40–49	97	22
	≥ 50	152	34.4
Gender	Male	283	64
	Female	159	36
Residence	Rural	141	32
	Urban	301	68
Marital status	Single	89	20.1
	Married	180	40.7
	Divorced	155	35.1
	Widow	18	4.1
Educational status	No formal schooling	61	13.8
	Primary school	109	24.7
	Secondary school	219	49.5
	Tertiary	53	12
Occupational status	Housewife	39	8.8
	Marchant	61	13.8
	Government employee	140	31.7
	Farmer	22	5
	Private employee	81	18.3
	Others [#]	99	22.4
Monthly income level (Ethiopian birr)	≤ 3000	121	27.4
	>3000	321	72.6

Others[#] - student, unemployed, daily laborer and self-employed.

Table 2. Clinical characteristics and laboratory investigations of HAART receiving HIV-positive study participants (n = 442) who were attending the ART clinic of Delgi primary hospital, Northwest Ethiopia (October 25th 2020 to June 19th 2021).

Clinical characteristics	Category	Frequency	Percentage (%)
Current BMI (kg/m ²)	<18.5	265	60
	18.5–25	100	22.6
	>25	77	17.4
Baseline BMI (kg/m ²)	<18.5	118	26.7
	18.5–24.99	205	46.4
	>25	119	27
Duration on ART (years)	≤5	319	72.2
	>5	123	27.8
Baseline WHO stage	Stage I	139	31.4
	Stage II	76	17.2
	Stage III	182	41.2
	Stage IV	45	10.2
WHO stage during the data collection period	Stage I	67	15.2
	Stage II	67	15.2
	Stage III	190	43
	Stage IV	118	26.7
Baseline opportunistic infection	Yes	45	10.2
	No	397	89.8
Current opportunistic infection	Yes	73	16.5
	No	369	83.5
History of chronic NCDs	Yes	29	6.6
	No	413	93.4
TB co-infection	Yes	133	30
	No	309	70
	No	309	70
HCV co-infection	Yes	33	7.5
	No	409	92.5
HBV co-infection	Yes	92	20.8
	No	350	79.2
Cotrimoxazole use	Yes	405	91.6
	No	37	8.4
Isoniazid preventive therapy	Yes	304	68.8
	No	138	31.2
Current adherence to ARV drugs	Good	186	42
	Fair	172	39
	Poor	84	19
Baseline adherence to ARV drugs	Good	129	29.2
	Fair	94	21.3
	Poor	219	49.5
Baseline regimen given	TDF-3TC-EFV	70	15.8
	D4T-3TC-NVP	122	27.6
	AZT-3TC-NVP	81	18.3
	D4T-3TC-EFV	106	24
	AZT-3TC-EFV	33	7.5
	TDF-3TC-NVP	30	6.8
ARV drug switching	Yes	233	52.7
	No	209	47.3
Switching ARV drug type	To the first-line drug	151	34.2
	To second-line drug	291	65.8
Reason for switching	Co-infection	71	16.1
	Pregnancy	26	5.9
	Drug toxicity	87	19.7
	Clinical failure	149	33.7
	Immunological failure	100	22.6
	Age	9	2

Table 2 (continued)

Clinical characteristics	Category	Frequency	Percentage (%)
Second regimen	AZT +3TC + NVP	89	20.1
	AZT+3TC + EFV	125	28.3
	TDF+3TC + NVP	63	14
	TDF+3TC + EFV	96	22
	ABC + ddl + LPV	45	10.2
	TDF + ddl + IPV	24	5.4
Current CD4 ⁺ T-cell count (cells/mm ³)	<200	42	9.5
	200–349	87	19.7
	350–499	133	30.1
	>500	180	40.7
Baseline CD4 ⁺ T-cell count (cells/mm ³)	<200	109	24.7
	200–349	76	17.1
	350–499	83	18.8
	>500	174	39.4
Current viral load count (copies/ml)	<1000	388	87.8
	≥1000	54	12.2
Baseline viral load count (copies/ml)	<1000	143	32.4
	≥1000	299	67.6
Reasons for ART eligibility criteria	Clinical staging	77	17.4
	CD4 ⁺ cell count	111	25.1
	Transfer in	19	4.3
	Test and treat	235	53.2
Duration since enrollment to eligibility (year)	<1	308	70
	≥1	134	30
Medication other than ARV drugs	Yes	86	19.5
	No	356	80.5
History of malnutrition	Yes	144	32.6
	No	298	67.4
Current hemoglobin level (g/dl)	<11.9	187	42.3
	>12	255	57.7
Baseline hemoglobin level (g/dl)	<11.9	301	68.1
	>12	141	31.9
Current functional status	Working	263	59.5
	Ambulatory	128	29
	Bedridden	51	11.5
Baseline functional status	Working	61	13.8
	Ambulatory	294	66.5
	Bedridden	87	19.7

BMI-body mass index, NCD-none communicable disease, ARV-antiretroviral drug, ART-antiretroviral therapy, WHO: World Health Organization, CD4: Cluster of Differentiation, d4T-stavudine, 3TC- lamivudine, NVP-nevirapine, EFV- efavirenz, AZT-zidovudine, TDF- tenofovir disoproxil fumarate, ABC- abacavir, ddl-didanosine, LPV- lopinavir.

(21.5%). Meanwhile, the age group ranges from 18 years to 72 years with a mean age of 37 ± 11.5 standard deviation (SD). In this study, 301 (68%) of the study participants were urban dwellers (Table 1).

13.2. Baseline information and clinical characteristics of study participants

The duration of HIV-positive participants on ART has revealed that 319 (72.2%) received ART for less than five years. Regarding the current WHO staging, about 190 (43%) study participants followed by 118 (26.7%) were at WHO stage three and four respectively (Table 2). In this study, the rate of current OIs 73 (16.5%) was relatively higher than the baseline history of OIs 45 (10.2%). Of the total study subjects, 133 (30%), 92 (20.8%) and 33 (7.5%) were co-infected with TB, HBV and HCV respectively. Antiretroviral drug switching has been observed among 233 (52.7%) study participants and 291 (65.8%) of them were in the second-line regimens. A total of 144 (32.6%) study subjects had a history of

Table 3. Bivariate and multivariate logistic regression analysis of the factors associated with the immunological failure among HAART receiving HIV-positive patients (n = 442) who were attending Delgi primary hospital, Northwest Ethiopia (October 25th 2020 to June 19th 2021).

Variables	Category	Frequency (%)	Immunological status		Binary logistic regression	Multiple logistic regression	p-value
			Success No (%)	Failure No (%)	COR (95%CI)	AOR (95% CI)	
Age (years)	18–24	95 (21.5)	83 (87.4)	12 (12.6)	1	1	-
	25–39	51 (11.5)	48 (94)	3 (6)	1.72 (1.04–8.04)	1.10 (0.50–2.01)	0.22
	40–49	213 (48.2)	197 (92.5)	16 (7.5)	0.84 (0.01–12.02)	0.95 (0.21–2.01)	0.97
	≥50	83 (18.8)	72 (87)	11 (13)	3.05 (1.75–7.52)	1.97 (0.02–4.03) *	0.01
Marital status	Single	89 (20.1)	82 (92)	7 (8)	0.12 (0.02–1.01)	1.02 (0.91–2.14)	0.62
	Married	180 (40.7)	156 (86.7)	24 (13.3)	1	-	-
	Divorced	155 (35.1)	150 (96.8)	5 (3.2)	4.19 (0.69–4.76)	0.23 (0.011–2.01)	0.82
	Widow	18 (4.1)	12 (66.7)	6 (33.3)	2.56 (0.45–5.09)	0.34 (0.10–2.56)	0.57
Current BMI (kg/m ²)	<18.5	265 (60)	247 (93.2)	18 (6.8)	1	1	-
	18.5–25	100 (22.6)	94 (94)	6 (6)	0.98 (0.06–2.11)	0.53 (0.21–1.23)	0.43
	>25	77 (17.4)	59 (76.6)	18 (23.4)	1.98 (1.07–3.02)	1.11 (0.60–2.22)	0.89
Duration on ART (years)	≤5	319 (72.2)	312 (97.8)	7 (2.2)	1	1	-
	>5	123 (27.8)	88 (71.5)	35 (28.5)	1.08 (1.01–2.55)	2.16 (0.90–3.33)	0.22
WHO stage during the data collection period	Stage I	67 (15.2)	60 (89.6)	7 (10.4)	1	-	-
	Stage II	67 (15.2)	65 (97)	2 (3)	1.90 (1.01–2.80)	-	-
	Stage III	190 (43)	180 (94.7)	10 (5.3)	2.50 (1.98–4.71)	-	-
	Stage IV	118 (26.7)	95 (80.5)	23 (19.5)	3.01 (0.95–12.06)	1.01 (2.37–8.40)	0.57
Current CD4 ⁺ T-cell count (cells/mm ³)	<200	42 (9.5)	0 (0)	42 (100)	1.26 (1.09–6.31)	2.16 (1.09–2.98)	0.33
	200–349	87 (19.7)	83 (95)	4 (5)	0.56 (0.02–2.51)	0.99 (0.25–1.88)	0.55
	350–499	133 (30.1)	124 (93)	9 (7)	1.11 (0.81–2.09)	1.44 (0.99–10.99)	0.66
	>500	180 (40.7)	168 (93.3)	12 (6.7)	1	1	-
TB co-infection	Yes	133 (30)	101 (76)	32 (24)	4.14 (1.18–16.01)	2.51 (1.02–7.51) *	0.05
	No	309 (70)	299 (96.8)	10 (3.2)	1	-	-
Current viral load count (copies/ml)	<1000	388 (87.8)	350 (90.2)	38 (9.8)	1	1	-
	≥1000	54 (12.2)	0 (0)	54 (100)	3.19 (2.59–7.71)	3.97 (1.09–5.01) *	0.03
Current hemoglobin level (g/dl)	<11.9	187 (42)	166 (88.8)	21 (11.2)	-	-	-
	>12	255 (57.7)	234 (92)	21 (8)	0.92 (0.04–1.98)	0.77 (1.02–9.08)	0.83

COR-crude odds ratio, AOR: adjusted odds ratio, * Significantly associated at p-value ≤0.05.

malnutrition and the majority 255 (57.7%) of their current hemoglobin level (g/dl) was greater or equal to twelve. Furthermore, the current hemoglobin level was too much smaller 187 (42.3%) than the baseline hemoglobin value of 301 (68.1%).

13.3. Immunological failure and associated risk factors among HAART receiving participants

In this study, the overall prevalence of immunological failure was 9.5% (42/442, 95%CI:3.23–15.09). In this study, the bivariate logistic regression analysis to address the risk factors associated with immunological outcomes was performed. In the analysis, variables with a p-value ≤ 0.2 were imported to multivariate logistic regression model analysis in order to control the confounding effect. As a result, study participants with age 50 years and above [AOR = 1.97, p = 0.01, 95%CI (0.02–4.03)], having viral load ≥1000 copies/ml [AOR = 3.97, p = 0.03, 95%CI (1.09–3.01)] as well as having TB-co-infection [AOR = 2.51, p = 0.05, 95%CI (1.02–7.51)] were statistically associated with increased risk of immunological failure (Table 3).

13.4. Virological failure and associated risk factors among HAART receiving participants

In this study, the overall prevalence of virological failure was found to be 54/442 (12.2%, 95% CI: 2.81–23.04). The multivariate logistic regression model analysis has revealed that TB co-infected HIV-positive participants receiving HAART were 1.88 at more risk to develop virological failure (AOR = 1.88, 95%CI = 0.89–10.02, p = 0.001) as compared to TB none co-infected individuals (Table 4).

14. Discussion

HAART has been improving the life of PLWHA by suppressing viral replication and boosting immune restoration [25]. The regular monitoring of immunological, and virological status, as well as the factors associated with poor immunological response and treatment outcome, has a significant role in providing improved quality of care, and overcoming the evolution/emergence of multiple drug-resistant viruses which limits the treatment option and increases HIV/AIDS-related complications such as morbidity and mortality rates among HIV/AIDS patients on HAART [10, 26, 31].

In this study, the overall prevalence of immunological failure was 9.5% (95%CI:3.23–15.09). The current finding is in line with previously conducted various studies including a systematic review and meta-analysis (n = 5,899) in 13 studies at the University of Gondar referral hospital [12] which reported 10.2% (95%CI 6.9–13.6), a study was done in Southern Ethiopia 11.5% [36], Jimma 9.8% [37], a study done in South Africa 11% [38] India 11% [39]. But, the current rate of immunological failure was slightly higher than the finding from the cross-sectional study conducted in the Tigray region [22] of Northern Ethiopia (n = 260 adults) which reports 6.5% immunological failure among HAART receiving participants, a cross-sectional study conducted in Liberia 5.1% [40]. However, the present prevalence was comparatively lower than the findings including a study done in Addis Ababa 15.7% [41] and 15% [42], and studies in the University of Gondar referral hospital 15.1 [43], 47.1% and 12.3% [33, 44] respectively, Kenya 64.4% [45], Tanzania 25% [46], China 18.4% [47], Colombia 14% [48], Thailand 33.5% [49], Nepal 35% [50], a prospective observational cohort (n = 158) study in the developing Caribbean country

Table 4. Bivariate and multivariate logistic regression analysis of the factors associated with the virological failure among HAART receiving HIV-positive patients (n = 442) who were attending Delgi primary hospital, Northwest Ethiopia (October 25th 2020 to June 19th 2021).

Variables	Category	Frequency (%)	Virological status		Binary logistic regression	Multiple logistic regression	
			Success No (%)	Failure No (%)	COR (95%CI)	AOR (95% CI)	p-value
Residence	Rural	141 (32)	107 (76)	34 (24.1)	1	-	-
	Urban	301 (68)	281 (93.4)	20 (6.6)	2.08 (0.99–13.01)	1.01 (1.11–5.55)	0.77
Duration on ART (years)	≤5	319 (72.2)	306 (96)	13 (4)	3.01 (5.06–6.07)	9.01 (92.33–14.04)	0.99
	>5	123 (27.8)	82 (66.7)	41 (33.3)	1	1	-
WHO stage during the data collection period	Stage I	67 (15.2)	54 (80.6)	13 (19.4)	1	1	-
	Stage II	67 (15.2)	61 (91)	6 (9)	2.07 (1.04–8.01)	-	-
	Stage III	190 (43)	176 (92.6)	14 (7.4)	0.56 (0.11–2.70)	1.23 (0.50–2.21)	0.88
	Stage IV	118 (26.7)	97 (82.2)	21 (17.8)	5.45 (0.86–6.01)	1.44 (0.22–3.33)	0.44
TB co-infection	Yes	133 (30)	125 (94)	8 (6)	2.7 (1.14–15.09)	1.88 (0.89–10.02) *	0.001
	No	309 (70)	263 (85.1)	46 (14.9)	1	-	-
HBV co-infection	Yes	92 (20.8)	61 (66.3)	31 (33.7)	0.83 (0.35–1.15)	2.08 (2.01–6.90)	0.26
	No	350 (79.2)	327 (93.4)	23 (6.6)	1	1	-
Current adherence to ARV drugs	Good	186 (42)	170 (91.4)	16 (8.6)	1	1	-
	Fair	172 (39)	153 (89)	19 (11)	1.37 (0.67–2.49)	0.70 (0.34–1.93)	0.82
	Poor	84 (19)	65 (77.4)	19 (22.6)	0.91 (0.01–2.14)	-	-
Baseline CD4 ⁺ T-cell count (cells/mm ³)	<200	109 (24.7)	101 (92.7)	8 (7.3)	1	1	-
	200–349	76 (17.1)	54 (71)	22 (29)	1.19 (0.61–1.93)	1.45 (0.09–10.03)	0.43
	350–499	83 (18.8)	75 (90.4)	8 (9.6)	2.67 (2.20–5.51)	1.56 (1.14–4.75)	0.37
	>500	174 (39.4)	158 (90.8)	16 (9.2)	0.85 (0.40–1.94)	1.37 (0.60–3.10)	0.49
Current viral load count (copies/ml)	<1000	388 (87.8)	255 (65.7)	133 (34.3)	1	-	-
	≥1000	54 (12.2)	0 (0)	54 (100)	4.32 (1.71–8.72)	3.07 (0.81–8.13) *	<0.001
Baseline viral load count (copies/ml)	<1000	143 (32.4)	108 (75.5)	35 (24.5)	4.54 (3.14–10.73)	2.71 (3.48–13.09)	0.66
	≥1000	299 (67.6)	280 (93.6)	19 (6.4)	1	-	-
History of malnutrition	Yes	144 (32.6)	111 (77)	33 (23)	2.11 (0.87–7.02)	1.62 (0.90–5.32)	0.31
	No	298 (67.4)	277 (93)	21 (7)	1	1	-
Current hemoglobin level (g/dl)	<11.9	187 (42.3)	158 (84.5)	29 (15.5)	1	1	-
	>12	255 (57.7)	230 (90.2)	25 (9.8)	2.57 (1.20–5.57)	1.37 (0.67–2.42)	0.36

COR-crude odds ratio, AOR: adjusted odds ratio, * Significantly associated at p-value ≤0.05.

21.5% [51] and a study in England 47.6% [52]. The variation in the magnitude of immunological failure may be explained due to the difference in the socio-economic conditions, the definition of immunological failure, the duration of HAART [22, 23, 25, 32, 33, 35, 43], medication adherence [26, 41, 53], study design, sample size and setting [26, 38, 54, 55], and immuno-virological discordance [15, 17, 18, 21, 38, 56]. Also, the discrepancy can be explained by the emergence of drug-resistance among HIV-positive patients [10, 16, 37, 40], their functional status [57], poor nutritional support [18], high burden of OIs [3, 11, 58], poor ART care, late initiation of HAART as well as ART-associated adverse reaction [23].

In the present study, study participants with age ≥50 years were 1.97 times at more risk to develop immunological failure as compared to the younger age groups [p = 0.01, 95%CI (0.02–4.03)]. In this age group, eleven out of eighty-three (13%) have shown immunological failure (p = 0.01). This finding agrees with the study conducted in Gondar [44] which stated that older (>40 years old) study participants had shown 47% less favourable immunologic status compared with the younger (age range of 26–40years old) age groups, a case-control study in Hawassa [26] stated that older age groups (fifty and above years old) were more likely to expose to immunological failure [AOR = 0.31, 95% CI [12-78] compared to younger groups. Besides, the present finding has shown similar findings to the previously conducted study which stated that participants having aged>50 years old were at increased risk of immunological failure [52, 55]. The effect of age on the immunologic restoration can be explained by younger age groups favours CD4⁺ cell restoration due to preserved thymus function which in turn contradicted the present study [59]. The discrepancy might attribute due to the ART adherence problem. A study revealed that the rate of poor adherence in

young individuals was higher than in older individuals [22, 60]. Furthermore, the older age group might have a better ART adherence which is the most critical to positively optimal the therapeutic outcome of HIV-positive patients [22, 44].

In the present study, TB-co-infection has been found an important influencing factor for immunological failure among HAART receiving study participants. Immunological failure was 2.51 times more likely to occur among TB co-infected HIV-positive individuals compared to TB non-infected individuals (p = 0.05). This was in agreement with previously conducted studies including from Gondar [61], Jimma [16], Ghana [62] and Nigeria [63]. This justifies that TB infection suppresses the cellular immune responses due to *M. tuberculosis*-induced apoptosis of CD4⁺ cells which in turn results in depletion of CD4⁺ T cells and subsequent immunological impairment [64, 65]. Another risk factor influencing immunological failure was the current viral load. HAART receiving study participants with a current viral load ≥1000 copies/ml were 3.97 times at a greater risk to develop immunological failure (p = 0.03) as compared to those who have <1000 copies/ml. This result was aligned with results reported from Ghana [62], Cambodia [23], India [66], Nepal [50], England [67] and the United States of America [68]. This might be due to increased viral replication and subsequent immunosuppression.

In this study, the overall virological failure among HAART receiving study participants was 12.2%. The current finding was similar to the reports from Mekelle 11.5% [22], Gondar 11.8% [33], Bahir Dar 10.7% [69] and Southern Ethiopia 15% [36]. Other previously done studies across the globe have shown similar findings of virological failure such as in Tanzania [70], South Africa [71], China and Cambodia [23] which report a prevalence of 12.3%, 13.7%, 13.4% and 12.9% respectively. The

present finding was comparatively higher than previously done studies including Jimma 5.3% [37], Gondar 4.1% [72] and Burkina Faso 7.5% [73] but, lower than the studies from Cameron, 23.2 [74], Kenya 24% [75], Liberia 47% [40] and Peru 24% [76]. The discrepancies might be attributed due to variation in the study population, follow-up period to HAART, study design, eligibility criteria, the definition of virological failure, medication adherence and nutritional support [10, 25, 33, 35, 72]. The difference may be also due to the type of interventions which was employed for the optimization of HIV-positive patients toward ART medications and the type of ARV regimen given up on their regular follow-up [15, 22]. In one study [73], protease inhibitors were given as the first-line regimen whereas, in another study, nutritional support was given [37] for the study participants which was quite different from the present study.

In this current study, the rate of virological failure was 1.88 times more likely to occur among TB-co-infected individuals compared with TB non-infect individuals ($p = 0.001$). This result was in line with the previously conducted reports from Uganda [77] and South Africa [78]. Virological no-suppression might be followed by TB Incident. Also, concurrent TB/HIV treatment can lead to impaired treatment adherence due to adverse drug interaction and the emergence of drug resistance [10, 25, 37] which can be considered a double burden.

15. Strengths and limitations of this study

This study addresses the risk factors associated with the poor immuno-virological response. Also, our study can provide better insight and reference by generating local epidemiological and clinical data for public health policymakers. Being a single-centred cross-sectional study, the outcome of the study may not represent a national figure. The research question was unable to address through a cohort study design with longitudinal data to account for the risk of treatment failure over time on therapy. Another limitation was that immune-virological discordance was not assessed.

16. Conclusion and recommendations

In this study, the overall prevalence of immunological and virological failure among HAART receiving HIV-positive study participants was found 9.5% and 12.2% respectively. Likewise, TB-coinfection was the single most important risk factor for both immunological and virological failure among study participants who were receiving HAART for at least six months. Evaluation of the immuno-virological status among HAART receiving HIV-positive study participants is a critical approach for systematically addressing the associated risk factors, provision of regular monitoring of therapeutic outcomes, and appropriate ARV regimen switching. Moreover, further intensive studies that provide a national picture in terms of clinico-epidemiological information regarding the immune-virological effect among people living with HIV/AIDS may be imperative to reverse the undesired therapeutic outcome.

Declarations

Author contribution statement

Teklehaimanot Kiros: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Abebe Taye: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Lemma Workneh: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Tahir Eyayu: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Shewaneh Damtie: Analyzed and interpreted the data; Wrote the paper.

Wasihun Hailemichael: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Tegenaw Tiruneh: Analyzed and interpreted the data; Wrote the paper.

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Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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