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COLLAGE OF HEALTH SCIENCE

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VIRAL SUPPRESSION RATE AND ASSOCIATED FACTORS AMONG CHILDREN WHOSE SAMPLE REFEREED TO ETHIOPIAN PUBLIC HEALTH INSTITUTE, ADDIS ABABA, ETHIOPIA.

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This is to certify that the thesis prepared by Gutema Bulti, entitled:

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List of Abbreviations

AIDS Acquired immunodeficiency syndrome

ART Antiretroviral therapy

EID Early Infant Diagnosis

EPHI Ethiopian Public Health Institute

HIV Human immunodeficiency virus

HAART Highly active antiretroviral therapy

MDGs Millennium Development Goals

NATs Nucleic Acid tests

PLWH People living with HIV

SDGs Sustainable Development Goals

UNAIDS United Nations Programme on HIV and AIDS

VS viral suppression

VL viral load

WHO World Health Organization

Abstract

Background: Measuring of Viral load in the plasma of patient is the most important indicator in response to Anti Retro viral treatment, and to monitoring regularly progress of the patient, by suppressing the Viral Load to a threshold of < 1000 RNA copies/ml, which is used to define suppressed viral load, if it is confirmed in the laboratory. adherence will be addressed and followed to switch to second-line Anti Retro viral treatment.

Objective: the aim of this study was to determine viral suppression rate and associated risk factors among children tested for HIV viral load among patient's sample refereed to Ethiopian public health institute, Addis Ababa, Ethiopia.

Method: Across-sectional study was conducted. Data on socio-demographic factors including Age, sex, test reason, treatment combination, adherence, treatment duration on Anti Retro viral treatment, WHO clinical staging, were extracted from the request, Viral suppression and failure was determined using the WHO definitions (viral suppression as viral load < 1000/ml) and their associated factors. Significant association between study variables and interpretation of data was done using the adjusted odds ratio (AOR) and 95% confidence interval and P value <0.05.

Result: A total of 340 children's sample was used in the study. Majority of children (87.9%) have suppressed viral load test rate and 12.1% non-suppressed. Children who had fair adherence to ART (AOR=0.23; 95% CI=0.001-0.395), were more likely to have viral load suppression rate. Children's whose treatment reasons were Routine VL-2ndVL at 12 Month Post ART, Routine Viral Load Annual Viral Load Test, Routine Viral Load-First Viral Load Test 6 month or Longer, Target Repeat (Confirmatory), Viral Load (initial viral load >1000cop/Ml) more likely had viral suppression rate (AOR=0.261;95% CI= 0.94-0.723), children between Age 3-10,were (AOR=17.76; 95% CI=1.623-194.19).

Conclusion The rate of un-suppressed viral load is High among children tested for HIV Viral Load in Ethiopian Public Health institute and Children adhered to ART treatment have more likely to be virally suppressed**.**

Keywords: HIV; antiretroviral therapy; adherence; stage of disease;

1.Introduction

1. 1.1 Background

Globally, 1.7 million people were newly infected with HIV which result in a total of 36.9 million people are living with HIV according to 2017 data. (1) Worldwide Around 220,000 children are, infected newly by human immunodeficiency virus (HIV), from this annually >50% are expected to die before age 2 years without antiretroviral treatment (ART), less than a third of children living with HIV have access to ART worldwide, Admittance to ART for children infected with HIV has increased subsequently from the beginning of millennium, in number from, 18 000 in 2000, and 823 000 in 2014 (2).

Africa is contributing more than two-thirds of new HIV infections, with 25.7 million HIV infected individuals. Only 40% of children with HIV received a test of Viral Load in Africa in 2012. Many studies have stated viral suppression rates in children on ART in low- and middle-income countries for the past years, however global summary estimate of long-term outcomes are underprovided so Measuring of VL in the plasma of patient is the most important indicator in response to ART and to monitoring regularly progress of the patient. (3, 4).

Virological states were defined as virological suppression (most recent VL below 50 copies/mL), low-level viremia (most recent VL between 51 and 999 copies/mL), and viremia (most recent VL ≥ 1,000 copies/mL) (5).

In comparison with adult's children exhibit exceptionally higher morbidity and mortality from HIV (6). This is because children acquire HIV due to their immature immune system, which leads to high rates of viral replication, high viral load, high rates of CD4 destruction, accumulation of mutations in the viral population and faster rates of disease progression (6, 7).

It is critical in children because sustained viremia reduces the impact of antiretroviral therapy (ART) for prevention and care of children health, but there has been inadequate implementation of viral load (VL) monitoring. In 2015 only 50% of HIV-infected children received ART in Low- and Middle-Income Countries, VS among children and adolescents remains far below that of high-income countries (8).

Virologic testing should be done at 4 to 6 weeks of age for all children who are exposed to HIV, as these children will need to be on Antiretroviral treatment as early as possible (9). In general

access to early testing for HIV-exposed children living with HIV has expanded; however, significant gaps exist across the testing to treatment cascade (10)., From estimated 34 million people living with HIV in 2011, 3.3 million were children under the age of 15 years (11). During the era of the Millennium Development Goals (MDGs), Intensive international solidarity and national efforts resulted in declining trends in AIDS-related deaths and new HIV infections (12). Based on the lessons learned from the MDGs, The Sustainable Development Goals (SDGs) aim to end the epidemic of HIV/AIDS by 2030 (13). This will result in a 90% decline in the number of new HIV infections and AIDS-related deaths between 2010 and 2030(12).

To translate evidence of ART's benefits from research studies into routine practice through a series of increasingly ambitious target setting initiatives The Joint United Nations Programme on HIV and AIDS (UNAIDS) and the WHO have encouraged countries and global partners (14,12,13). UNAIDS launched the 90-90-90 target in 2014: With the goal of ending the AIDS epidemic by 2030. by 2020, 90% of PLWH know their HIV status; 90% of all people with diagnosed HIV infection are on ART and 90% of people accessing treatment have suppressed VL. Mathematical modeling suggests that front-loading resources to reach 90-90-90 by 2020, with a further increase to 95-95-95 and the scale-up of other prevention measures by 2030, can reduce new HIV infections and AIDS deaths worldwide by 90% between 2010 and 2030 (9,10,15).

Achievements according to UNAIDS 90-90-90 target by 2020, 75% of PLWH knew their status (approximately three out of four people), 79% of them (approximately four out of five HIV positive patients that knew their status) were accessing treatment and among those accessing treatment, 81% (approximately four out of five HIV positive patients that had started ART treatment) were virally suppressed. The Joint UNAIDS ‘90-90-90’ targets require the use of VL monitoring to assess the ‘third-90’ (14, 12).

The third ‘90’achievement is mainly attributed to the WHO recommendation of Test and Treat approach in that ART should be initiated in all PLWH regardless of their WHO clinical stage and Viral Load (16), proper administration of ART therapy through the 4S (Start, Substitute, Switch and STOP) ART medicine being free and it takes much ease for both the health center and patients to easily coordinate and start ART treatment (17, 18).

The advantage of early ART for Children with HIV infection is to completely suppress viral replication, thus preventing further damage to the immune system, decreasing AIDS-associated morbidity and mortality, allowing immune function to return to normal, and reducing the risk of transmitting HIV infection to others (19,20).

1.2 Statement of Problem

The success in saving lives has not been matched with equal success in reducing new HIV infections. New HIV infections are not falling fast enough. Children are being left behind (13).

One of the challenges to achieving optimal treatment outcomes for children in resource limited setting is late initiation of ART (21, 22). Routine laboratory monitoring of HIV VL is not performed in the public treatment Programme. Because of this Children with advanced disease at ART initiation are less likely to achieve VS and have higher rate of mortality and morbidity in comparison with adults. Although, countries have expanded early infant diagnosis (EID) services, many children continue to be missed and are enrolling late to care (10). Decisions regarding initiation or changes in antiretroviral therapy are guided by monitoring plasma or DBS HIV RNA levels (viral load), and the patient's clinical condition (23). In addition to late enrolment, differences have also been observed in rates of suppression by age at ART initiation. Several studies have shown that time to VS is often prolonged in infants and younger children and those infants are less likely to completely suppress (10, 24). Achieving VS protects the body's immune system, helps Children stay healthy and prevents transmission of HIV to other people (7, 25). children in Ethiopia are often required to take a number of tablets each day Due to the limited availability of pediatric fixed-dose ARV formulations, which may well contribute to this poorer adherence (7). The cost of inaction will be huge-if countries do not scale up HIV prevention and treatment services rapidly by 2020, but instead continue with the existing coverage levels of services, they will lose the opportunity to save 21 million lives, and an additional 28 million people would be living with HIV by 2030. Continuation of current coverage levels will mean that the world will have to pay an additional US$ 24 billion every year for ART by 2030 (12, 26).

Nevertheless, with additional 4.0 million PLWH recorded in 2017, the Eastern and Southern Africa countries could be confronted with huge challenge to achieving the third 90% (27). Ethiopia is one of the high burden countries in the sub–Saharan African constitutes about 80% of the total human immunodeficiency virus (HIV) infections worldwide. The overall prevalence of HIV infection is around 1% (2.9% urban, and 0.4% rural (28). Moreover, there are 62,000 (38,000-86,000) children younger than 14 years living with HIV; and annually nearly 5,500 (2,600-8,800) new HIV infections occur in children in 2017 (14, 29).

Apart from the recent Ethiopian Population-based HIV impact assessment; most of the studies were conducted before the implementation of the "test and treat" strategy. Some children may take ART, but these drugs may not control the HIV replication. Poorly controlled HIV can be due to many factors, including lack of health care, poor medication adherence, incomplete drug absorption, drug resistance and drug toxicity. Therefore; the study was focused on identified gaps like Adherence, treatment regimen, socio demographic related factors, and other factors that can affect the children viral load suppression status, in order to provide evidence to the identified gaps and to forward recommendations.

1.3 Significance of the study

Once viral suppression is achieved, it is of importance to know whether suppressed patients are likely to be able to maintain VS and which factors are associated with VS. The percentage of HIV-positive persons with durable VS provides a helpful indicator for monitoring national HIV management priorities and guiding prevention and treatment efforts. Additionally, the durability of VS will help define the best timing of targeted adherence strategies and intensive VL monitoring in individuals with multiple challenges to ART adherence.

Identification of predictors of virological failure is important for health programmers since it can enable better targeting of adherence support. This analysis of factors associated with VS in Ethiopia thus aims to identify groups at risk of having non-suppressed VL and hence virologic failure, to enable better targeting of adherence support and referral for clinician review.

In Ethiopia, most of the studies were focuses on adult populations; this made data on VS among children in Ethiopia scare as only very few institutional and regional based studies conducted in different part of the country. Generally, no adequate national, regional and institutional figure on VS rate among children tested for HIV VL in Ethiopia. In addition to this there was limited evidence on risk factors associated with un-suppressed viral load in infants in Ethiopia. Thus, this study was aimed to determine Viral suppression rate among children tested for HIV viral load, and associated risk factors. Moreover, I believe that the result of this study will be used for the initiation of further research to control VS rate among children HIV patient as general population.

2 Literature Review

Monitoring VL nowadays, it is the best standard for evaluating effectiveness of ART and risk of transmission in PLWH (30). Determining the number of VL in plasma is best indicator of ART response and treatment monitoring. In the study conducted by Reepalu A et al, plasma VL suppression was classified as suppressed (if VL is < 1000 copies/ml) and unsuppressed (if VL is > 1000 copies/ml) in the plasma (3).

Effective Highly active antiretroviral therapy (HAART) significantly decreases the number of mortality and morbidity of children infected by HIV (31,21). The right HAART, immunizations and prophylactic antimicrobials for PLWH have been demonstrated to substantially reduce death and prevent hospitalizations. Good adherence to ART and retention in care will bring success to ART. High VS is the best for prevention of viral failure, decreases viral transmission and HIV/AIDS-related problems (32,33).

Initiation to ART in sub-Saharan Africa was a hot issue because of many challenges about adherence, logistics supplies and resistance. Nowadays, it has been significantly increased. According to the WHO clinic-immunological approaches for initiation and monitoring of ART in the region, sub-Saharan Africa countries lacks VL determination and drug resistance monitoring access, HIV infected Children may be at risk by developing both virologic failure and antiretroviral drug resistance (20).

A recent research from US showed that older patients were more likely to achieve VS (34). Both children and adolescents treated by ARV faced with several challenges including the complexity in ARV dosage and dose adjustment as the children grow, which may be difficult for providers who are not skilled with pediatric care or too busy to track the suppression status (35).

Other study conducted in Swaziland on Children and adolescents were indicated a detectable VL, and less re-suppressed at retesting. This may be related to the limited available evidence on VL monitoring in children, and brings the specific barriers to adherence for children and adolescents (36).

In sub-Saharan Africa there is a challenging in achieving VS for children. Studies reported that children and adolescents have lower VS rate as compared to adults in Kenya (57% to 66% vs. 63% to 87%) and in other developing countries (60% -75% vs. 85%), when compared to children

and adolescents in developed countries (≥ 90%). Many factors may influence adherence and VS in children such as age, familial and socioeconomic environment, stigma, disclosure, and the physical and mental health status of children (22,37).

Developing countries could be achieved the WHO target of 90%. A study done in Uganda showed that the prevalence of the non-suppressed VL was 11% among HIV infected children (38), this is less when compared to the suppression rate of the study conducted in Bahirdar (9). other developing countries, such as Cameroon has also reported level of VL< 80%. Prevalence of VS can be varied between both age groups; the recorded prevalence of virological failure is high among adolescents (46.7%) (VS ≥ 1000 copies/mL), when compared to that of children (24.4%) (39).

On the basis of study conducted in Ghana showed, regular attendance to ART (adherence to medication) and HAART initiation for more than three (3) years were associated with the rate of VS (7).Other studies in Ghana indicated that, from 195 HIV infected patients achieved VS, 77 (39.5%) had undetectable VLs (<20 copies/ml), similarly (39.5%) achieving VL results on the basis of cut-off values of 20-200 copies/ ml (27,40).

In study conducted by Assefa et al, it was indicated that Ethiopia is almost on the way to achieve the fast-track targets (HIV testing, ART, viral suppression) by implementing SDG. However, only achieving the target is not enough to tackle the epidemic of HIV, as Ethiopia is still left back in reduction of new HIV infections, stigma and discriminatory attitudes, and HIV prevention (13).

On the basis of study conducted in Bahidar, from total of 1567 children tested for viral load, the VS rate was about 71.7%. Out of 1567 participants, 28.3% (444/1567) had non-suppressed HIV VL. From children referred for routine VL testing 27.1% developed viral failures (VL results >1000 copies/ml). About 24.3% (18/74) children <5 years were reported as high viremia (6).

Other studies conducted at Eastern Oromia showed that, 13% of participants enrolled in the study have a plasma VL of <10,000 copies/μl. VLs in patient's plasma were suppressed in 72% for those linked to ART treatment (41).

A high VL (>1000 copies/ml) in a patient on ART for at least 6 months can be due to either therapeutic failure (resistance to ART), or poor adherence to medication (9). Routine VL

monitoring for HIV-infected children are considered as a priority group than others. Viral failure is defined when a threshold of VL is > 1000 RNA copies/ml. After the viral failure is confirmed, intensive adherence should be fixed well, and followed by linking to second-line ART (6). A patient with an elevated VL should receive adherence support followed by retesting after 3 to 6 months. Patients whose VLs are not suppressed at retesting can be stated as developing 'virologic failure' (WHO guidelines), it can be probable drug resistance, and should be linked to second line therapy for success treatment (9).

There is high prevalence of VS failure in suspected of anti-retroviral drug failure as compared to the prevalence among children diagnosed for routine VL and it is associated with VS (6). Adherence to treatment is one of the important factors that influences treatment outcome. VL is not suppressed in poor adherence, this is leads to opportunistic infections, drug resistance and ultimately death (18, 26).

Socio-demographics and baseline clinical characteristics will be used as exposure variable (19, 41). The association of clinical condition of children at initiation of ART and treatment adherence is not well developed. Resistance to ART drug is a problem because once it develops resistance; the patients can no further use that drug to reduce their VL. Poor adherence to medication and mutation can bring drug resistance (26). The golden standard for HIV treatment monitoring is VL testing by using Abott m2000 rt automated machine, which could indicate the amount of HIV genetic material (RNA) circulating in the blood plasma. In addition to VL study it is better if the sequencing of the genetic material and HIV drug resistance genotyping for better achievement of required target (23,42).

3. Objective

3.1. General Objective

- To determine Viral Suppression rate and Associated Factors among Children attending HIV viral load testing at EPHI Addis Ababa Ethiopia, 2021

3.2. Specific Objective

- To determine Viral Suppression rate among children attending HIV viral load testing at EPHI.
- To determine the associated factors of Viral Load among children attending HIV viral load testing at EPHI.

4. Materials and Methods

4.2. Study area

The study was conducted on sample which referred from Oromia region, southern part of Ethiopia and from different health sector of Addis Ababa and the test is performed in Addis Ababa city Administration, Ethiopian Public Health Institute. Addis Ababa is rapidly expanding city with a total land area of 54,000 hectares 540sq.km. It had an administrative structure of 10 sub-cities and 99 kebeles. According to the 2007 census, the total population of Addis Ababa is 4,793,699, 47.64% (1,304,518) males and 1,433,730(52.36%) females. EPHI is located in Addis Ababa Gulalle sub city Arbegnoch Street (the former Pasteur Institute): with area of 30.18 sq.km and total population of 284,865 from these 147,175 females and 137,690 males. EPHI is performing referral lab service and National HIV Reference laboratory is one Directorate which was performing HIV VL, Qualitative EID testing with PCR, hepatitis B and C VL, PT production for EID and VL testing laboratories, and currently performing COVID-19 testing and also HIV drug resistance. The laboratory was built by CDC and have well established quality systems and accredited to ISO 15189; 2012 by ENAO.

4.3 Study period

The study was conduct from February 2021 G.C to June 2021G.C

4.1. Study Design

A cross-sectional study design was used.

4.4 source and study populations

4.4.1 Source population

Source of population was all samples of people living with HIV and attend viral load testing in Ethiopian Public Health Institute at National HIV Reference laboratory during the study period.

4.4.2 Study population

The study population was, all sample of children with age lower than 15 years who was accessed HIV viral load testing service in the EPHI during the study period.

4.5 Eligibility criteria

4.5.1 Inclusion criteria

- Samples of Children who referred for VL test and have at least for one-year data at EPHI HIV reference laboratory.
- Samples of Children with complete requisition and adequate plasma samples.

4.5.2 Exclusion criteria

Requests with missing age and poor specimen quality such as hemolysis was excluded

4.6. Sample Size and sampling technique

4.6.1 Sample size

Taking prevalence of VLS rate (72%) (10), from a study conducted in Addis Ababa Ethiopia was used and sample size was calculated using a single population proportion formula;

$n = (Z\alpha/2)2\ (p*q)/\ d^2$

Where: n = sample size

$Z\alpha/2$ = level of confidence =1.96,

d= margin of error

$$n = \frac{(1.96)^2(0.72*0.28)}{(0.05)^2} = 310$$

Considering, 10% non- response rate, the total sample size was 340.

4.6.2. Sampling technique

All samples that fit the inclusion criteria was collected until sample size was fulfilled using convenient sampling technique.

4.7 Study variables

4.7.1 Dependent variable

- Viral suppression rate

4.7.2 Independent variables

- age
- sex
- test reason
- treatment combination
- Adherence
- Treatment duration on ART
- WHO clinical staging

4.8. Data collection tools and Procedures

4.8.1. Specimen collection.

In this study, the sample was not collected from the children's/guardian directly since the specimens were collected from the peripheral health facilities. When postal couriers and/or vehicle drivers and other referring sites submitted samples at the central reception of the EPHI, specimen quality and completeness of requests was checked, then Each child specimen was coded using bar code, all this was done after ethical approval and official permission was obtained from Addis Ababa University ethical committee and EPHI to use the viral load data in the laboratory.

4.8.2. Data Extraction process.

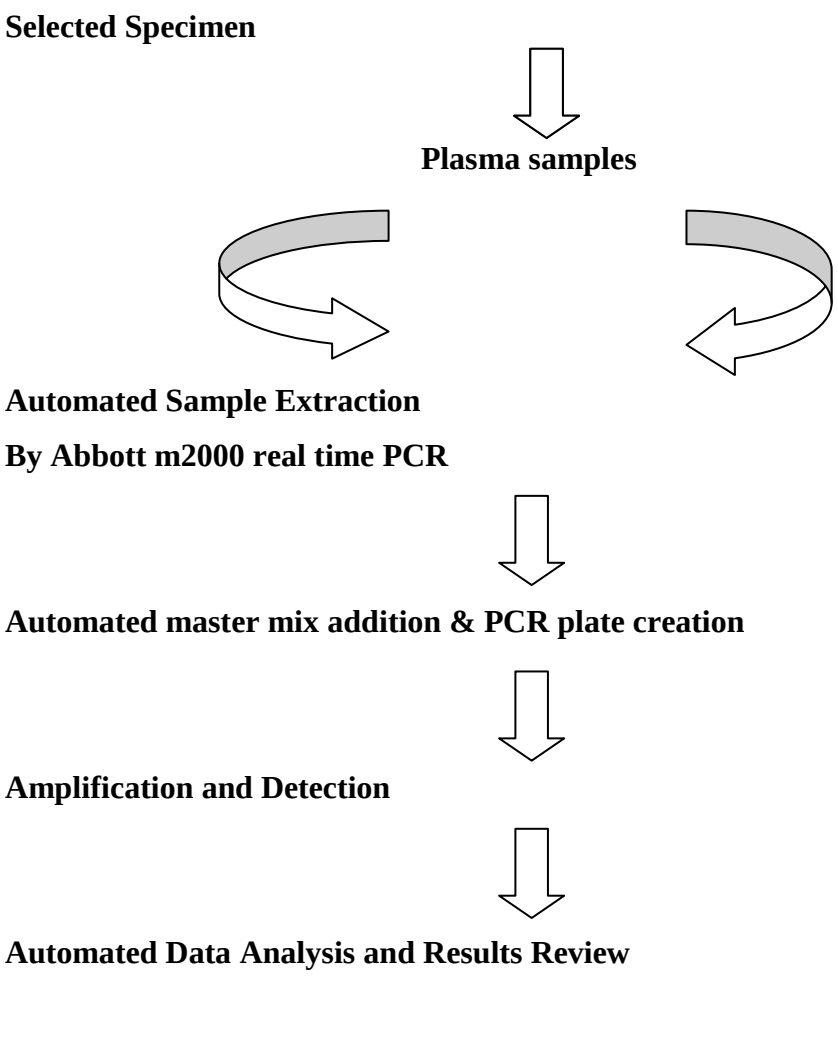
The data extraction tool was prepared by the investigator and used to collect data after pre checked. Data of Children's who have one base line viral load test result within one year at EPHI HIV reference laboratory was extracted by PI and data clerics. Variables of interest were collected from the requests and go to collect the base line viral load result from EPHI excel data base. Socio-demographic and clinical data was extracted from the laboratory requests using a data retrieval checklist on spot is cross checked with the first base line data base and collect on excel sheet. Then all collected selected specimen is directly submitted to the HIV molecular reference laboratory for VL testing.

4.8.3. Testing for Viral load testing status

Trained laboratory analysts were investigated the VL test following the standard operating procedure (Annex I). Nucleic acid tests (NATs) which include HIV RNA and HIV DNA PCR assays were a key to diagnose HIV infection in infants (20). It was done using advanced molecular techniques. In brief, nucleic acid extraction was done from the children's plasma

samples using an automated m2000sp machine (Abbott Molecular inc. USA). Extraction reagents were used as follows: M lysis to destruct membrane; magnetic particle to trap RNA; mwash1 and mwash2 to purify the nucleic acid; and elusion buffer to break the bond between magnetic particle and the RNA. The unrelated RNA sequence was also introduced into each specimen at the beginning of sample preparation as an internal control to demonstrate that the process proceeded correctly for each sample: Negative, low positive and high positive controls were used in each test order to evaluate run validity. Then, the extracted nucleic acid was mixed with amplification reagents (thermo stable polymerase enzyme in buffered solution, oligonucleotides, quencher, reference dyes and activation reagent) to amplify and detect HIV1 RNA using an automated m2000rt machine (Abbott Molecular inc., USA). After laboratory analysis, results were interpreted as not detected and detected (number of HIV virus RNA copies). The lower and higher detection limit of the machine is 150 and 10,000,000 RNA copies per ml, respectively based on the manufacturer's procedures (Annex II). Results with greater than 1000 HIV virus RNA copies per ml was considered as not suppressed viral load results (40, 41).

Figure 1Diagram of sample process



4.9. Data management

The confidentiality of the data collected was kept to the maximum as each patient identity was coded. Every detail patient information was used only for the above-mentioned research purpose and was not be given to third part in any form.

4.10. Quality assurance

The data was collected by cross checking between me and data cleric and incomplete data was excluded. The data quality was checked and monitor throughout the data collection, entry and analysis process (preanalytical, analytical and post analytical stage of quality assurance was included)

Preanalytical

All the request come with referred Sample was cross checked by trained laboratory personnel according to inclusion and exclusion criteria putted.

Analytical

Sample fulfilled inclusion criteria was performed depend on Standard operating procedure. Maintenance, start up and sample extraction procedure of Bio-safety cabinet, vortex is performed with 0.1 % bleach followed by distilled water and finalized with 70% ethanol before and after the work. UV light used for 15 minutes. Abbott m2000 sp instrument Log on to the software by entering user ID and password Perform all necessary maintenance including daily, weekly and as needed maintenance. A minimum of one Negative Control and one Positive Control are required with each run.

post analytical

Trained Data clerk and PI insert the result by using barcode to keep data quality and confidentiality of the result on laboratory information system to minimize clerical error and result directly send to central reception.

4.11. Data analysis

Data was analyzed using SPSS version 20. Descriptive statistical analysis s was used to describe demographic and clinical characteristics of the study population. The association between outcome variable and independent variables were measured using backward likelihood ratio

(LR) method in the multivariable logistic regressions. Multivariable models were adjusted for all variable characteristics. Significant association between study variables and interpretation of data was done using the adjusted odds ratio (AOR) and 95% confidence interval and *P* value <0.05 was considered as significant association.

4.12. Operational definitions

Adherence - Adherence to a medication is generally defined as the extent to which patients take medications as prescribed by their health care providers. In HIV treatment, adherence < 95% is associated with virologic failure, opportunistic infections and deaths.

Viral suppression - meaning the levels of HIV in a person's blood (viral load) are so low that the laboratory test cannot measure them.

Detectable - Number of HIV virus RNA copies

Viral suppression rate – The rate of viral load for Children's who are on ART at least for 1 year or who's their viral load is done two times every six months with viral load result below 1000copies/ml.

Fair Adherence: 85%-94%.

Poor Adherence: <85%

Good Adherence: >95%

4.13. Ethical consideration

Before the initiation of actual data collection, ethical approval was obtained from the Addis Ababa University department of Medical Laboratory sciences Ethical Review Committee. After support letter was written from the ethical clearance committee to the EPH institution, permission to use the available data was sought from the institution.

5. RESULT

5.1 Socio-demographic characteristics of study participant

Socio demographic factors of the children whose samples were collected 340(100%) shows that; majority of children's 187(53.8%) were male and 238(70) of them were from Addis Ababa region. Regarding age of children 216(63.5%) was between 10-15 years followed by 97(28.5%) which was among 3 to 10 years.

Among other factors viral load status of children show that 299(87.9%) of children shows suppressed viral load rate while 41 (12.1%) were non-suppressed. Regarding treatment reason majority285 (83.8%) of children were attending treatment for Routine Viral Load Annual Viral Load Test. The WHO clinical staging of children participated in study show that 323(95%) of children were in stage one.

Table1. socio demographic characteristics of study participant at EPHI, Addis Ababa, Ethiopia, 2021. (N=340).

Variable	Number	Percent
Socio demographic characteristics		
Sex of children		
Male	183	53.8
Female	157	46.2
Total	*340*	100.0
Region of children		
Addis Ababa	95	27.9
Oromia	238	70.0
SNNPR	7	2.1
Total	340	100.0
Age of children		
between 0-3 years	27	7.9
between 3 to 10 years	97	28.5
between 10-15 years	216	63.5
Total	340	100.0

Table 1 factors associated with viral load suppression rate at EPHI, Addis Ababa, Ethiopia, 2021. (N=340).

Factor associated with VL		
Viral load suppression status		
Suppressed	299	87.9
Non suppressed	41	12.1
Total	340	100.0
Test reason		
Routine VL-2ndVL at 12 Month Post Art	5	1.5
Routine Viral Load Annual Viral Load Test	285	83.8
Routine Viral Load-First Viral Load Test 6 month or Longer ART	23	6.8
Target Repeat (Confirmatory)Viral Load (initial viral load >1000cop/ml)	22	6.5
Target suspected ART failure	5	1.5
Total	340	100.0
WHO clinical staging		
Stage I	323	95.0
Stage II	17	5.0
Total	340	100.0
ART regimen		
First Line	323	95.0
Second Line	17	5.0
Total	340	100.0
Adherence		
Fair (85%-94%)	28	8.2
Good (>95%)	260	76.5
Poor (<85%)	52	15.3
Total	340	100.0
Treatment combination		
(TDF+3TC+DTG 4j/(ABC+3TC+DTG	158	46.5
AZT+3TC+LPV/r/ABC+3TC+LPV/r	130	38.2
AZT-3TC-EFV/TDF-3TC-EFV	52	15.3
Total	340	100.0

Table2 below showing socio-demographic and other variables Associated with Viral load suppression rate among children on follow up for viral load testing at EPHI, Addis Ababa, Ethiopia, 2016. (N=340).

Factors associated to VLS rate among children's sample collected and tested at EPHI in binary logistic regression. In binary logistic regression sex of child (p-value=0.103), Region of children in Addis Ababa (p-value=0.045) and Oromia(p-value=0.006), routine viral load annual viral load test treatment reason (p-value=0.010), being good or fair adherence to Treatment (p-value=0.00) respectively, children on (TDF+3TC+DTG/ (ABC+3TC+DTG (p value=0.242), children in age category of between 3 to 10years (p-value=0.001) years were variables associated with children's viral load suppression rate. Table (2)

Table 2Factors Associated with viral load suppression rate at EPHI, Addis Ababa, Ethiopia, Ethiopia, 2021. (N=340)

Variable	Viral load suppression rate status					
	suppressed N (%)	**Non-supp. N (%)**	**(95% CI) COR**	**P-value**	**(95% CI) AOR**	**p-value**
Sex of children						
Male	**143 (42.1)**	14 (4.1)	1			
Female	156 (45.9)	**27 (7.9)**	**1.768 (.892-3.504)**	**0.103**	**0.015(0.001-0.332) ***	**0.008**
Age category						
between 10-15 years	198 (58.2)	18 (5.3)	**3.227 (1.639-6.351)**	**0.001**	**17.76 (1.623-194.19) ***	**0.018**
between 3 to 10 years	75 (22.1)	22(6.5)	**1**			
between 0-3 years	26(7.7)	1 (0.3)	**0.423 (0.054-3.302)**	0.412	0.00(0.00-)	0.998
Region of children						
SNNP	4 (1.2)	3 (1.2)	1			
Addis Ababa	89 (26.2)	6 (1.8)	**0.207 (.044-.969)**	**0.045**	**0.001(0.001-)**	**0.990**
Oromia	206 (60.6)	32 (9.4)	**0.009 (.016-.497)**	**0.006**	**0.001(0.001-)**	**0.990**
WHO Clinical staging						
Stage II	0	17(5)	**1615475293.5(0.00-0.0)**	**0.998**		
Stage I	299(87.9)	24(7.1)		**0.998**		

ART Regimen						
Second line	15(4.4)	2 (0.6)	1.00			
First line	284 (83.5)	39 (11.5)	**1.030** (0**.227**-**4.676**)	0.97**1**		
Treatment reason						
Routine viral load first-viral load 6 months or longer on ART	17(5)	6(1.8)	1			1
Routine VL-2ndVL at 12 Month Post Art	4(1.2)	1(0.3)	0.708 (0.66-7.659)	0.776	0.000(0.000-.)	0.992
routine viral load annual viral load test	261 (76.8)	24 (7.1)	0.261 (0.94-0.723)	0.010	0.000(0.000-.)	0.992
Target repeat (confirmatory) viral load (initial viral load)>1000cop/ml	13(3.8)	9(2.6)	1.962 (0.556-6.918)	0.295	0.000(0.000-.)	0.992
Target suspected ART failure	4(1.2)	1(0.3)	0.708 (0.66-7.659)	0.776	0.000(0.000-.)	0.992
ADHERANCE						
Poor (<85%)	13(3.8)	39(11.5)	1			
Fair (85%-94%)	27(7.9)	1(0.3)	0.012(0.002-0.10)	0.00	0.23(0.001-0.395) *	0.009
Good (>95%)	259 (76.2)	1(0.3)	0.001 (0.00-.010)	0.001	0.001(0.001-)	0.988
Treatment combination						
AZT-3TC-EFV/TDF-3TC-EFV	44(12.9)	8 (2.4)	1		1	
(TDF+3TC+DTG/ (ABC+3TC+DTG	143 (42.1)	15 (4.4)	0.577(.229-1.451)	.242	1.004(125-8.088)	0.997
AZT+3TC+LPV/r/ABC+3TC+LPV/r	112 (32.9)	18 (5.3)	0.884(.358-2.180)	0.789	2.654(0.303-23.281)	0.378

P-value <0.25 candidates for multivariate,

6. DISCUSSION

The study reveals 87.9% children were observed with suppressed viral load and 12.1% unsuppressed. This may indicate, a high VL (>1000 copies/ml) in a patient on ART for at least 6 months can be due to either therapeutic failure (resistance to ART), or poor adherence to medication (16). This result in line with the study conducted on the difficulty of achieving VS rate in sub-Saharan Africa (25,38) and also with the study conducted in Kenya which was 57% to 66% when compared to developed countries which is ≥ 90% VS rate (25,38). This could be due to the difference in factors like socio-economic, stigma, physical and mental health status of children. Also, A high VL (>1000 copies/ml) in a patient on ART for at least 6 months can be due to either therapeutic failure (resistance to ART), or poor adherence to medication (16).

Also, when comparing the VS status among study samples, from those who has suppressed viral load 88.6 % of them has undetectable viral suppression status while 11.4 has detectable viral load status which was less than 150 copies/ml. this is higher when compared with study conducted in Ghana that indicated, from 195 HIV infected patients achieved VS, 77 (39.5%) had undetectable VLs (<20 copies/ml), similarly (39.5%) achieving VL results on the basis of cut-off values of 20-200 copies/ ml (9,39)

The result of viral load suppression rate found in this study was higher when compared to the viral suppression rate of the study conducted in Bahirdar which was about 71.7 % (13). The result of viral suppression rate in this study was also almost similar when compared to other studies conducted in developing countries like Uganda with non-suppressed VL was 11% among HIV infected children while 12.1 in our study (37).

The observed result of our study showed that children 10-15 year also can be classified as (adolescent based on latest treatment category) age groups were 17.76 times more likely to have viral load suppression rate compared to Childs of between age 0-3years. But results of others studies showed that the Prevalence of VS rate among age group the recorded of virological failure is high among adolescents (46.7%) (VS ≥ 1000 copies/mL), when compared to that of children (24.4%) (38); the other study in sub-Saharan Africa also supports that there is a challenging in achieving VS for children. Studies in Kenya reported that children and adolescents have lower VS rate as compared to adults (57% to 66% vs. 63% to 87%). The difference could be due to study area, and population definition as our country defines children

are between 0-15 (42) while in other countries define those among 10-15 years as adolescent. This study also tried to identify certain socio demographic factors associated with viral suppression rate; Accordingly, the study shows that Female children were 1.78 times more likely to have viral suppression rate compared to male; this result supports the explanation that indicated that; "Socio-demographics and baseline clinical characteristics will be used as exposure variable" (18,40).

Also, the result of this study shows that adherence to ART has association with viral load suppression. This shows effective highly active antiretroviral therapy (HAART) has significantly contributed to VL suppression rate which helps in decreasing the number of mortality and morbidity of children infected by HIV (20, 30). Also, the right HAART, immunizations and prophylactic antimicrobials for PLWH have been demonstrated to substantially reduce death and prevent hospitalizations (21, 36). Accordingly in our study; a child with fair adherence to ART (≥85% adherence to ART) was 0.23 times more likely to have suppressed viral load status. This result in line with the study conducted in Ghana which showed regular attendance to ART (adherence to medication) and HAART initiation for more than three (3) years were associated with the rate of VS (7). Beside it is evident that many factors may influence adherence and VS in children such as age, familial and socioeconomic environment, stigma, disclosure, and the physical and mental health status of children (21, 36).

7. Conclusion and recommendations

7.1 Conclusion

The study showed that 87.9 % of children have viral suppression rate and while 12.1% have un-suppressed viral load. Also, Children adhered to ART treatment 85% and more have more likely to be viral suppression rate. Even though the finding of this study is relatively higher than other studies conducted in Ethiopia, the proportion viral load suppression rate was still found to be low or not optimal to WHO target greater than 90%.

7.2. Recommendation

1. As our finding indicates adherence is significantly important to reduce the viral load among children on ART. Therefore, the government, non-government organization, and other stake holders could focus on strengthening the holistic approach to HIV care and treatments through awareness creations using all effective mechanisms like mass media and health education specifically targeting adherence to ART and mainly for families of children with HIV positive status.

2. The policy makers should address this issue as a matter of policy as there is a need to promote awareness viral load suppression; such policies should address by including roles of all stakeholder's importance of their involvement and its outcomes.

8. Limitation of the study

This study had some limitations it comes up with; as our study population was sample referred for VL test and some variables were collected from client's request paper. For that reason, this study did not assess variables like family income, nutritional status of children, family awareness about HIV viral load, co-infections like bacteria or parasite.

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6. Annexes

I. Procedure for HIV-1 Quantitative PCR from Plasma using ABBOTT m2000sp/rt

Quality Control

Control	Level	Stability	Frequency	Preparation (yes/no)
Kit control	High/low positive	-20^{o}C until expire date	Every run	No
Kit control	Negative	-20^{o}C until expire date	Every run	No

Note: A minimum of one Negative Control and one Positive Control are required with each run.

Detailed Procedure

Part I: Maintenance, Start up and sample extraction procedure

Bio-safety cabinet and vortex Maintenance

Clean the hood with 0.1 % bleach followed by distilled water finalized with 70% ethanol before and after the work and use UV light for 15 minutes.

Clean centrifuge and vortex with 0.1 % bleach, distilled water followed 70 % ethanol

Startup procedure

Turn on Abbott *m*2000System Control Center (SCC) and Abbott *m*2000 sp instrument

Log on to the software by entering user ID and password.

Initialize the Abbott *m*2000sp instrument by click on initialize, after initialization complete the Abbott sp instrument is ready for operation in ready mode

Perform all necessary maintenance including daily, weekly and as needed maintenance

Reagent preparation

For up to 24 reactions use: one tube of High and low positive control, one tube of Negative Control, one Amplification Reagent Pack, and one set of *m*Sample Preparation System*RNA*reagents.

For 25 to 48 reactions use: one tube of Positive Control, one tube of Negative Control, two Amplification Reagent Packs, two bottles of *m*LysisBuffer, and one bottle *m*Wash1 Buffer, *m*Wash2Buffer, *m*Microparticleand*m*ElutionBuffer.

For 49 to 72 reactions use: one tube of High and low Positive Control, one tube of Negative Control, three Amplification Reagent Packs; three bottles of *m*LysisBuffer; two bottles of *m*Wash1Buffer and *m*Wash2Buffer; and one bottle of *m*Microparticleand *m*ElutionBuffer.

For 73 to 96 reactions use: one tube of High and low Positive Control, one tube of Negative Control, four Amplification Reagent Packs; four bottles of *m*LysisBuffer; two bottles of *m*Wash1 Buffer and *m*Wash2Buffer; and one bottle of *m*Microparticleand *m*ElutionBuffer.

Thaw assay controls and IC at 15-30°C or at 2-8°C.

Once thawed, assay controls and IC can be stored at 2-8°C for up to 24 hours before use.

Gently mix each assay control three times for ≈ 3 seconds.

Thaw amplification reagents at 15-30°C or at 2-8°C and store at 2-8°C until required for the amplification master mix procedure.

Once thawed, the amplification reagents can be stored at 2-8°C for up to 24 hours if not used immediately.

Use one bottle of *m*Lysis buffer, one vial of IC

Invert gently the Abbott System RNA Preparation *bottles* to ensure a homogeneous solution without generating any bubbles.

Ensure bubbles or foam is not generated; if present, remove with a sterile pipette tip, using a new tip for each bottle.

Open the *m*Sample Preparation System*RNA*reagent pack(s).

If crystals are observed in any of the reagent bottles upon opening, allow the reagent to equilibrate at room temperature until the crystals disappear.

Do not use the reagents until the crystals have dissolved.

Vortex each IC vial three times for 2-3 seconds before use.

Add 750 µL of IC to each bottle of *m*Lysis Buffer.

Mix by gently inverting the container 5 to 10 times to minimize foaming.

Pre-extraction procedure

Prepare sample and generate the work sheet

Carefully check all plasma samples. Avoid of bubbles and clot in the samples.

Place the high positive controls, low positive control and negative control and the patient specimens into the Abbott *m*2000*sp* sample rack.

Place the 5 mL Reaction Vessels into the *m*2000*sp* 1 mL subsystem carrier

Load the Abbott *m*2000 Sample Preparation System reagents and the Abbott 96 Deep-Well Plate on the Abbott *m*2000*sp* worktable as described in the Abbott *m*2000*sp* Operations Manual, Operating Instructions section

Select the appropriate application file from the Run Sample Extraction screen that corresponds to the sample volume being tested.

Initiate the sample extraction protocol as described in the Abbott *m*2000*sp* operations manual, operating instruction section

Enter control lot specific values in the Sample Extraction: Worktable Setup and Control fields. The Abbott *m*2000*sp* Master Mix Addition protocol must be initiated within one hour after completion of Sample Preparation.

Master Mix Addition

Load the amplification reagents and the master mix vial on the *m*2000*sp* worktable after sample preparation is completed. Each Amplification Reagent Pack supports up to 24 reactions.

Ensure that the contents are at the bottom of the vials prior to opening the amplification reagents by tapping the vials in an upright position on the bench.

Remove and discard the amplification vial caps.

Select the appropriate deep well plate from the Run Master Mix Addition screen that matches the corresponding sample preparation extraction.

Initiate the Abbott *m*2000*sp* Master Mix Addition protocol.

Follow the instructions as described in the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.

The *m*2000*rt* protocol must be started within 40 minutes of the initiation of the Master Mix Addition protocol.

Switch on and initialize the Abbott *m*2000*rt* instrument in the amplification area which requires 15 to warm-up.

Seal the Abbott 96-well optical reaction plate

Export completed PCR plate results to a CD or by internet and import to Abbott *m*2000*rt* instrument/enter manually the data

Place the sealed optical reaction plate into the Splash Free Support Base for transfer to the Abbott *m*2000*rt* instrument.

Make sure the Abbott *m*2000*rt* instrument has been initialized

Place the Abbott 96-Well Optical Reaction Plate in the Abbott *m*2000*rt* instrument.

From the protocol screen selected the appropriate application file corresponding to the parameter and volume being tested

Initiate the protocol as described in the Abbott *m*2000*rt* Operations Manual, Operating Instruction section

Note; Summary of reagent amount used for extraction

Samples	1st Vessel	2nd Vessel	3rd Vessel	4th Vessel	5th Vessel	6th Vessel

1-24	one *m*Lysis	N/A	One *m*Microparticle	One *m*Wash1	One *m*Wash2	One *m*Elution
25-48	Two *m*Lysis	N/A	One *m*Microparticle	One *m*Wash1	One *m*Wash2	One *m*Elution
49-72	Two *m*Lysis	One *m*Lysis	One *m*Microparticle	Two *m*Wash1	Two *m*Wash2	One *m*Elution
73-96	Two *m*Lysis	Two *m*Lysis	One *m*Microparticle	Two *m*Wash1	Two *m*Wash2	One *m*Elution

Interferences/Limitations

Optimal performance of this test requires appropriate specimen collection, handling, preparation, and storage.

Result Reporting

HIV-1 Detected result will be reported as number of viral load

HIV-1 not Detected result will be reported as Negative.

Results Interpretation

A specimen tested by the Abbott Real T*i*me HIV-1 Quantitative assay will have a result of "HIV-1 with its viral load" or "Not Detected (undetectable)"

References

Abbott RealTi*m*e HIV-1 Quantitative kits insert.

Abbott *m*2000*rt* operational manual.

Data extraction format

Data extraction format for a research topic viral suppression rate and associated factors among children whose sample referred to Ethiopian public health institute, Addis Ababa, Ethiopia.										
MRN	**Variables**	Age	sex	treatment reason	Treatment Combination	Adherence	WHO clinical staging	Region	First Viral Load Result	second viral load result
0001										
0002										

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: **Gutema Bulti (B.Sc.)**

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisors:

Mr. Melese Hailu (BSc, MSc, PhD fellow)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: **Mr. Shambel Araya (BSc, MSc)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.