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Incidence and predictors of Tuberculosis among children on antiretroviral therapy in Amhara region, Ethiopia

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Abstract:

Introduction: Tuberculosis (TB) continues to be a significant public health issue, particularly in low- and middle-income countries. Globally, the end-TB strategy targets an 80% reduction in TB incidence by 2030. Despite this strategy, there is limited evidence on the incidence of TB among HIV-infected children in the study area after the test and treatment strategies in Amhara region. Hence, this study aimed to assess the incidence of TB and its predictors among HIV-infected children receiving antiretroviral therapy in the Amhara region.

Methods: A multicenter institution-based retrospective follow-up study was conducted on 421 HIV-infected children receiving antiretroviral (ART) treatment from July 2014 to March 2022. The study participants were selected using a simple random technique. National antiretroviral intake and follow-up forms were utilized to gather data through the KoBo Toolbox. STATA version 17 was used for data analysis. The Kaplan-Meier curve was applied to estimate failure time, and the log-rank test was employed for comparison among groups of predictors. To find TB predictors, Gompertz regression models, both bivariable and multivariable, were constructed. Ultimately, a 95% confidence interval-adjusted hazard ratio was calculated, and variables with a p-value less than 0.05 were considered statistically significant.

Results: A total of 421 children with a record completeness rate of 97.9% were analyzed in the study. The tuberculosis incidence rate in children on ART was 2.16 (95% CI: 1.52, 3.05) per 100 child-year observations. Those children who were on baseline anemic [AHR: 3.83 (95% CI: 1.46, 10.04)], never took TB preventive treatment (TPT) [AHR: 3.78 (95% CI: 1.44, 9.94)], wasted children [AHR: 2.53 (95% CI: 1.19, 5.38)], and not initiated antiretroviral therapy within seven days [AHR: 2.35 (95% CI: 1.15, 4.78)] were the significant predictors.

Conclusion: The incidence of TB in children on ART was relatively high. HIV-positive children presenting with anemia, who never took TPT, wasted, and late initiation of ART were prone to the occurrence of TB. Therefore, needs emphasis on prompt treatment of anemia and TB preventive therapy, counseling on nutritional improvement, and timely initiation of ART to avert TB.

Keywords: Antiretroviral therapy; HIV-infected children; incidence; predictors; test and treat Tuberculosis

Introduction

Globally, tuberculosis (TB) is a primary cause of morbidity and mortality, and it's a global emergency in all regions and affects every country in the world. Globally, TB incidence varies , with 30 high-burden countries accounting for 87% of cases [1]. Children and young adolescents (age below 15 years) account for 1.25 million (12%) of the total TB incidence across the globe every year [2].

TB remains an important public health problem, particularly in low and middle-income countries (LMIC) [3]. The risk of developing this global infectious disease is 18 times higher among HIV-infected individuals [4]. In addition to increasing the suffering of infected individuals, it remains the second leading cause of death from infectious disease, and the risk of death is threefold higher among HIV-infected individuals worldwide [3, 4].

Recently, the global occurrence of TB mortality in children increased significantly, which was 214,000 in 2023. Each day, 600 children lost their lives to this preventable disease. Approximately 31,000 deaths are related to people with HIV [1, 5]. In Sub-Saharan African countries, the HIV-TB burden has increased, despite the test and-treat policy in this region. The incidence rate of tuberculosis among HIV-infected children is 3.42 per 100-person year [6]. This problem has a significant economic burden in low- income ad middle- income countries [3]. Like in other Sub-Saharan countries, in Ethiopia, tuberculosis is a significant health problem among HIV-infected children, with an incidence of 4.33 per 100-person year [7].

The occurrence of tuberculosis (TB) among children on ART treatment is 24.2% to 32% in Nigeria [8-10], and 15.2% in Ethiopia [11], while the incidence of TB among children with HIV varies from 0.83 to 2.3 in China [12], 7 per 1000 person-years in Thailand [13], 4 in South Africa [14], , 2 to 2.63 in Ethiopia [15-17], and 17.4 in East Africa [18].

Some factors contribute to the causes of TB among children on ART treatment, such as extra-pulmonary TB, severe immunosuppression, age less than two years, and WHO HIV clinical stage [19-21]. Underweight [21], a low Cluster of Differentiation 4 (CD4) () count, anemia [22], and virological failure [23] also contribute to the occurrence of TB among children on ART treatment. To better understand the variety of contexts and the application of new approaches in factors that

affect the risk of tuberculosis (TB) in children living with HIV, additional investigation is necessary.

The achievement of Ethiopia's Sustainable Development Goals (SDG s) and the end-TB strategy targets established by the World Health Organization (WHO) and the United Nations, which aim to reduce tuberculosis cases by 80% and deaths from tuberculosis by 90%, provide support for this study [2, 24]. The political declaration of the UN high-level meeting on TB held in WHO plans to reach TB Preventive Treatment (TPT) at least 90% of people with HIV in 2027 [25].

Ethiopia has adopted a test-and-treat strategy since June 10, 2014, for all children initiation of ART regardless of CD4 cell count and WHO clinical staging aiming at reduction of HIV-related mortality and morbidity [26]. Despite this intervention, there is paucity of evidence of the overall incidence of TB in children enrolled in the Amhara region after the test and treat strategy. In addition to previous studies, this finding covers a wide range of public institutions, and in this study, rapid initiation of ART and doultegravir (DTG)-contained drugs were incorporated as independent variables to the determine incidence of TB. Timely and recent evidence is very crucial. Therefore, by incorporating these new variables and new strategy needs further investigation. The finding will provide input for policy-makers and decision-makers on HIV/AIDS care and support at various levels. Therefore, this study aimed to assess the incidence of TB and its predictors among HIV-infected children enrolled in ART in the Amhara Region.

Methods

Study Design, setting, and period

A multicenter institution based retrospective follow-up study was conducted among HIV-positive children enrolled at Amhara region comprehensive specialized hospitals from July 2014 to March 2022. The Amhara region is located in the Northwestern, Northeastern and North-central parts of Ethiopia, with an estimated area of 159,173.66 square kilometers. The most recent estimated population in this region is 30,848,988 [27]. In this region, there are 81 Hospitals; among those, eight are comprehensive specialized and teaching hospitals, the University of Gondar, Tibebe-Ghion, Felege Hiwot, Debre Markos, Debre Tabor, Dessie, Woldia, and Debre Birhan.

Those hospitals have been providing ART care and support as part of the National AIDS Control Program. Among the total number of Comprehensive Specialized Hospitals, seven were included

in the study area, except Tibebe-Ghion, due to the inadequate study population. A total of 1095 children were newly enrolled in HIV care in the Amhara region from July 2014 to March 2022.

Population and eligibility

All HIV-positive children ages less than 15 who were enrolled in the pediatric HIV care clinic in Amhara Region Comprehensive Specialized Hospitals and had at least one month of ART follow-up were the source population. HIV-positive children aged less than 15 years who were enrolled in the pediatric HIV care clinic in Amhara Region between July 2014 and March 2022 were the study population. Children with HIV who already had TB before starting ART and records with incomplete baseline information (CD4 count, WHO staging, hemoglobin level, weight, and height) and unknown dates of ART initiation and TB occurrence were excluded from the study.

Sample size determination

The sample size was calculated using common significant predictor variables (height for age, WHO clinical staging, and ART adherence level). STATA version 17 and the cox proportional hazard model were used for sample size determination through the following assumptions: $Z\alpha/2$ = is the critical value of a standard normal distributed variable at 5% significance level = 1.96; $Z\beta/2$ = is the critical value of a standard normal distributed variable at 20% = 0.84; the probability of an event was 0.167 [28]; and the probability of withdrawal was 0.15 using an adjusted hazard ratio (AHR). (**Supplementary table 1)**

Sampling procedures, and sampling technique

From the calculated sample sizes, the largest sample size (430) was selected as a sample for this study. The sample was allocated proportionally to those comprehensively specialized hospitals in the Amhara region, and records were selected using simple random techniques. **(Supplementary Figure 1:)**

Variables of the study

The outcome variable for this study was the occurrence of TB during the follow-up period. The independent variables were socio-demographic characteristics: age, sex, residence, current parent's status, educational status of the caregiver, HIV disclosure status, and marital status of the caregiver. Baseline clinical, nutritional, and laboratory characteristics: CD4 count, WHO clinical staging, hemoglobin level, anthropometric indices, previous OIs, functional and developmental

status. ART and medication-related characteristics: baseline ART regimen, DTG containing ART drugs, presence of regimen change, level of ART adherence, TPT, CPT, ART side effects, initiation of ART.

Operational definition of the variables

Events: The occurrence of TB among HIV-infected children during the follow-up period at any time after enrollment in the pediatric HIV care clinic, as confirmed by a health care professional. **TB cases** were diagnosed based on the national TB diagnosis guideline, using sputum or gastric aspirate microscopy, chest X-ray examination, and/or histopathology.

Children, who were lost to follow-up, dropped out, transferred out, died due to any causes, or completed the study period before developing TB considered as a censored.

TB-free probability time was considered between ART starting and the TB diagnosis date.

Wasting: if the weight for height (WFH) Z-score is less than -2 SD for less than five years, or if the body mass index (BMI) for age Z-score is less than -2 SD for greater than five years [29].

The level of adherence to ART: good adherence is reported with compliance equal to or greater than 95% or ≤ 3 missed doses per month as documented by the ART health personnel; fair reflected 85–94% compliance and between 4 and 8 missing doses per month) as documented by the ART health personnel; and poorly reflected less than 85% compliance or ≥ 9 missed doses per month) as documented by the ART health personnel [29] .

Rapid initiation of ART: ART initiation care and support on the same day of HIV confirmation or within seven days [30].

Anemia was defined as having a hemoglobin level of less than 10 mg/dl [31].

Data collection tool and procedures

The data were collected retrospectively from the ART intake form, follow-up form, and children`s charts using the data extraction tool adopted from Ethiopian ART guidelines newly registered at ART clinics during the period of July 2014 to March 2022 [29]. Data was extracted for one month, from May 17 to June 15, 2022. The variables consist of socio-demographic, clinical, laboratory, ART, and medication-related variables. Data were collected by seven bachelor's degree nurses

who had smartphones and who were familiar with the ART follow-up and taking basic ART training.

Data quality control

The data extraction tool was pretested on 5% of the sample size before the actual data collection period at the University of Gondar (UoG) Comprehensive Specialized Hospital. Moreover, one-day onsite training was given on how to review ART follow-up and medical records, data collection methods, and the objective of the study for data collectors and supervisors. Data were collected using the KoBo toolbox, which was prepared with relevant restrictions by trained nurses working in Hospitals. Besides, the data collector and principal investigators carefully monitored the entire data collection process and daily submission report.

Data processing and analysis

The KoBo toolbox was used, which was then exported and imported into STATA version 17 statistical software for final analysis. Additionally anthropometric indices were created using the WHO anthro and WHO anthroPlus software. To describe the data, descriptive statistics including mean (±SD), percentage, and frequency were employed. The Variance inflation factor (VIF) was used to check the association between predictor variables; the average VIF was found to be 1.18, it indicates no significant multicollinearity**.**

A separate graph of Kaplan-Meier survival functions and the log-rank test were estimated for each categorical variable to compare survival between different exposure groups. The proportional hazard assumption (PHA) was checked using both graphical and statistical global tests and revealed that the PHA was satisfied. The log-likelihood and Akaike Information Criteria (AIC) were applied to select the best-fitted model, and a model with a minimum AIC was considered the best-fitted model. Based on this, the Gompertz regression model with the lowest value (AIC = 236.02) was the best-fitted model. In addition, the goodness-of-model fitness was also checked using the Cox-Snell residual test. Variables having a p value less than 0.25 in the bivariable analysis were fitted into the multivariable Gompertz regression model. A hazard Ratio with a 95% CI was used to determine the strength of the association. Variables with a $p<0.05$ in multivariable analysis were considered statistically significant.

To handle and manage missing data, multiple imputations using multivariable chained equations (MVCE) were carried out to handle and manage missing data. The variables missing at random (MAR) are clarified by the "Little test" results for continuous variables and graphical patterns for categorical variables, making the application of multiple imputations straightforward. Furthermore, sensitivity analysis was performed utilizing both descriptive and inferential statistical techniques to confirm if a significant difference was detected between the outputs of the original and imputed data.

Patient and public involvement

Patients and public were not directly involved in the design, conduct and dissemination of this study.

Results:

1. Socio-Demographic characteristics

A total of 430 medical records of HIV-infected children on ART were reviewed. Of these, 421 (97.9%) were included in this study, and the remaining percent were excluded from the analysis due to data incompleteness. The mean (SD) age of the study participant was 7.58 (±4.02) years; one-third was found in the age group of 5-9 years. Nearly two-thirds (58.43%) were male, and 70% were from urban areas. **(Table 1:)**

Table 1: Socio-demographic characteristics of incidence of Tuberculosis among HIV-infected children on ART at Amhara Regional State Comprehensive Specialized Hospitals, Ethiopia, 2022 (n=421)

Variables	Categories	Frequency(n)	Percentage (%)
Age			
	<5 Years	122	28.98
	5-9 Years	145	34.44
	10-14 Years	154	36.58
Sex			
	Female	175	41.57

	Male	246	58.43
Residence			
	Rural	126	29.93
	Urban	295	70.03
Current parent status			
	Both parents alive	290	68.88
	One parent alive	113	26.84
	Both parents deceased	18	4.28
Educational Status of the caregiver			
	No formal education	144	34.2
	Primary	150	35.63
	Secondary	67	15.91
	Tertiary (College & above)	60	14.25
Marital status of the caregiver			
	Married	228	54.16
	Unmarried	87	20.67
	Divorced	70	16.63
	Widowed	36	8.55
HIV status of the parents			
	Non-reactive	158	37.53
	Reactive	263	62.47
Disclosure status the child			
	Yes	220	52.26
	No	201	47.74

2. Baseline clinical, laboratory, nutritional and ART medication related characteristics

At baseline, 79.9% of children were in WHO clinical stages I or II. The median CD4+ count was 421 (IQR: 403, 1017) cells/l, and one fifth (20.19%) had CD4+ below the threshold level. The median hemoglobin level was 12.3 (IQR: 10.7, 13.6) mg/dl, and 17.34% had less than 10 mg/dl hemoglobin level. Nearly 30% and 50% of children were wasted and stunted at baseline, respectively. Out of 421 HIV infected children, 296 (70.31%) had a good ART drug adherence level. More than two-thirds (63.42%) of children had never received TPT, and 45.13% of children never taken ART within seven days of its duration. **(supplementary table 2:)**

3. Follow-up time and incidence of TB incidence

A total of 421 children followed from 1 to 89 months. The mean follow-up time was 42 months (14.57 to 70.07 months). The total time at risk was 1484.75 child-year observations (CYO). New TB cases were observed in 32 (7.6%) children; the overall TB incidence rate was 2.16 (95% CI: 1.52, 3.05) per 100 CYO. **(Supplementary figure 2:)**

The incidence rate of TB was 3.98 (95% CI, 1.99, 7.95) per 100 CYO in the six months of ART initiation and decreased to 0.33 (95% CI, 0.05, 2.34) per 100 CYO after three years of ART initiation. The cumulative probability of TB incidence at the end of two, four, and six years of the study was 0.9636, 0.9309, and 0.9045, respectively. The incidence rate of TB was 8.09 (95% CI, 5.03, 13.01) per 100 CYO among anemic children, while it was 1.18 (95% CI, 0.7, 1.95) per 100 CYO among non-anemic children. **(Figure 1)**

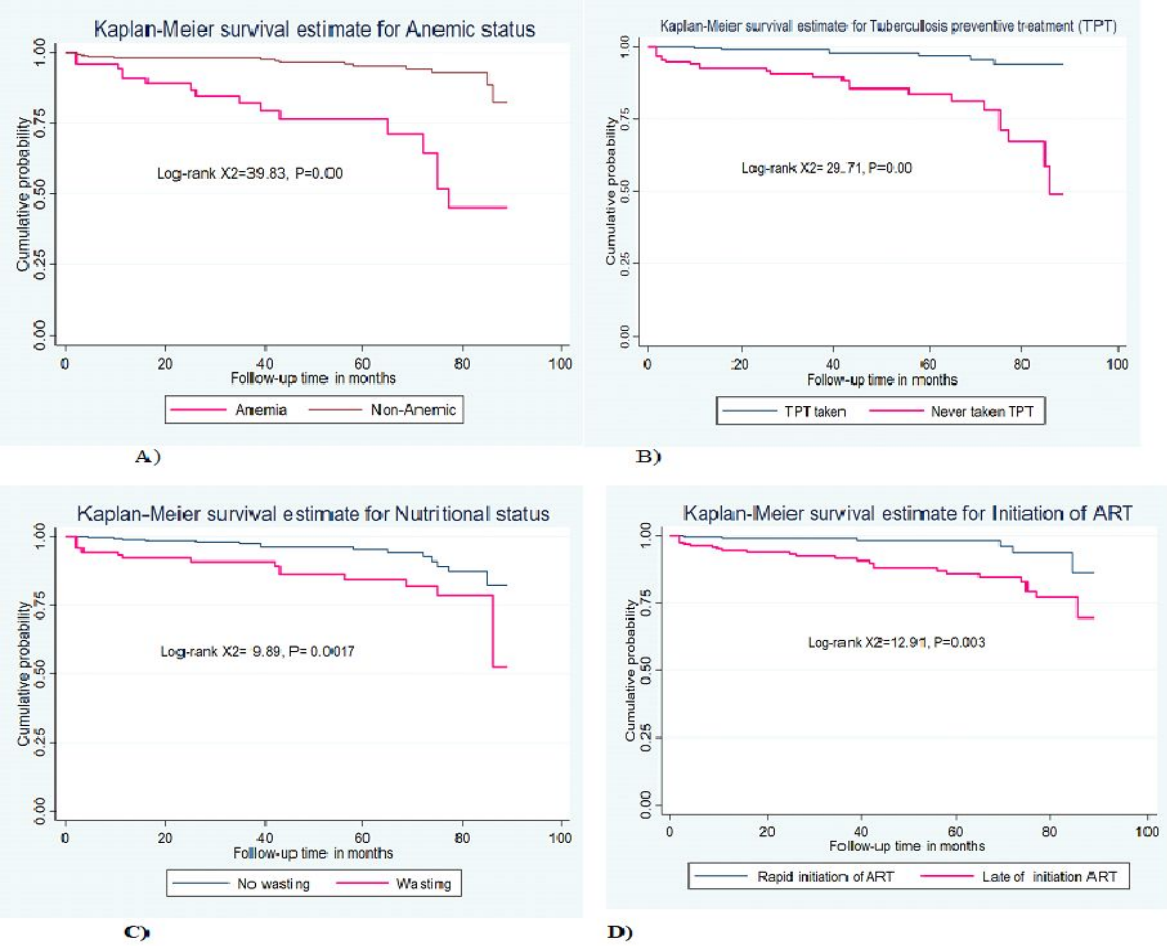

Figure 1: Kaplan-Meier of TB free survival of anemic status (A), tuberculosis preventive therapy (B), nutritional status (C), and initiation of antiretroviral therapy (D) main predictor variables among children on ART in Amhara region comprehensive Specialized Hospitals

4. Model comparison

The proportional hazard assumption, semi-parametric, and parametric proportional hazard models were fitted to estimate the incidence of tuberculosis and its predictors among HIV-infected children in the Amhara region. The graphical test and global test (P-value = 0.2980) confirmed the proportional hazard assumption (PHA). The most fitted model was chosen using Akaike

information criteria (AIC = 236.02), Bayesian information criteria (BIC = 304.74), and log-likelihood (-138.43). The results outperformed the Cox-proportional hazard and other parametric models in all three comparison techniques. As a result, interpretations and conclusions were based on the Gompertz model. **(Supplementary table 3 :)**

The Cox-Snell residual test checked the goodness of fit. **(supplementary figure 3)** The shared frailty was estimated, (theta: $1.89e^{-07}$), and the distribution of unmeasured variables is indifferent between the comprehensive specialized hospitals.

5. Predictors of incidence of Tuberculosis

In bi-variable parametric survival Gompertz regression: parental status of the care givers, educational status of the care givers, baseline of WHO clinical staging, CD4+ count, hemoglobin status, functional and developmental status, regimen change, ever taking TPT, ever taking CPT, ART adherence level, previous opportunistic infections, doultegravir (DTG) contained ART drug, wasting, and rapid initiation of ART were found to be significant predictors of TB among children in ART. In the final multivariable Gompertz regression model, only baseline hemoglobin, ever taking TPT, wasting, and rapid initiation of ART significantly increased the incidence of tuberculosis.

Anemic children had 3.83 times [AHR: 3.83 (95% CI: 1.46, 10.04)] higher risk of getting TB infections than those in non-anemic children. Likewise, the risk of developing TB for children who were not given TPT was 3.78 times [AHR: 3.78 (95% CI: 1.44, 9.94)] higher as compared with those who were given TPT at baseline. Additionally, the hazard of TB among children with wasted children at baseline was 2.53 times [AHR: 2.53 (95% CI: 1.19, 5.38)] more likely as compared to their well-nourished counterparts. Furthermore, children had late ART initiation 3.27 times [AHR: 3.27 (95% CI: 1.26, 8.46)] increased risk of developing TB compared to rapid initiation of ART **(Table 3).**

Table 3: Bi-variable and multivariable Gompertz cox-regression analysis of predictors of incidence of Tuberculosis among HIV-infected children on ART in Amhara Region Comprehensive Specialized Hospitals, Ethiopia, 2022 (n = 421).

Variables	Status		CHR (95% CI)	AHR (95% CI)	P value
	Event (n=32)	Censored (n=389)			
Parental status of the child					

Both parents alive	19	271	**1**	**1**	
One or both parents dead	13	118	1.77 (1.88, 3.59)	0.8 (0.32, 2.02)	0.64
Educational status of the caregiver					
No formal education	9	135	1.27(0.49, 3.29)	0.72(0.25, 2.04)	0.53
Primary	15	117	2.05(0.87, 4.83)	0.97(0.33, 2.81)	0.96
Secondary and Tertiary	8	137	1	1	
WHO Clinical staging					
Stage I & II	17	328	**1**	**1**	
Stage III & IV	15	61	5.47 (2.73, 11.00)	1.71(0.62, 4.71)	0.299
CD4 cell count					
Above threshold	21	315	**1**	**1**	
Below threshold	11	74	2.89(1.39, 6.02)	1.59(0.59, 4.31)	0.357
Anemia					
No	15	333	**1**	**1**	
Yes	17	56	7.26(3.62, 14.56)	3.83 (1.46, 10.04)	0.006
Working and appropriate motor developmental status					
Yes	16	267	**1**	**1**	
No	16	122	2.39(1.19, 4.8)	1.66(0.77, 3.61)	0.196
Adherence level of ART					
Optimal	16	280	**1**	**1**	
Sub-optimal	16	109	2.79(1.39, 5.59)	0.92(0.36, 2.4)	0.874
ART regimen change					
Yes	15	199	**1**	**1**	
No	17	190	1.63(0.81, 3.27)	0.77 (0.32, 1.87)	0.57
Ever taking tuberculosis preventive treatment(TPT)					
Yes	7	260	**1**	**1**	
No	25	129	7.55(3.26, 17.47)	3.78(1.44, 9.94)	0.007
Ever taking cotrimoxazole preventive therapy(CPT)					
Yes	21	324	**1**	**1**	
No	11	76	2.68(1.29, 5.57)	1.28(0.5, 3.27)	0.601
Weight for height					
No wasting	15	284	**1**	**1**	
Wasting	**17**	**105**	**2.9(1.45, 5.81)**	**2.53(1.19, 5.38)**	**0.016**
Previous opportunistic infections					
No	17	306	**1**	**1**	
Yes	15	83	3.43(1.71, 6.88)	1.32 (0.54, 3.19)	0.54
Doultegravir (DTG) contained drugs					
Yes	6	63	**1**	**1**	
No	26	326	0.46 (0.18, 1.14)	0.37 (0.13, 1.03)	0.059
Initiation of Antiretroviral therapy					
Rapid Initiation	6	**225**			
Late Initiation	**26**	**164**	**4.41(1.81, 10.74)**	**3.27(1.26, 8.46)**	**0.015**

**** Significant at α 0.05, 1: reference, *CHR, crude hazard ratio, AHR, Adjusted hazard ratio***

Discussion

This study aimed to assess TB incidence rate and its predictors in children on ART in Amhara region comprehensive specialized Hospitals. In this study, HIV-infected children on ART were 2.16 (95% CI 1.52, 3.05) per 100 child-years observations(CYO), which is consistent with study conducted in Debre Markos 2.63 [32], in Northeast Ethiopia 2.0 [28], and Southern Ethiopia 2.6 per 100 CYO[33]. This finding is lower than study conducted in Northern Ethiopia [34], in Benishangul Gumuz region in Northwest Ethiopia [35, 36], in Adama Referral Hospital and Medical College [37], in South west Ethiopia [38], and in Tanzania [39]. This could be due to higher burden of tuberculosis in resource limited settings[40], and also might be due to the difference in sociodemographic and baseline clinical characteristics of the study participant, changes in the study period and setting.

However, our study is higher than studies conducted in New York City [41], in Ireland and the United Kingdom [42], and in China [43]. This could be because the general population in high-income countries has a low incidence rate of tuberculosis. In addition, the use of early TB diagnosis and prevention techniques, along with the accessibility of modern technologies, significantly lowers the incidence of tuberculosis [44]. Furthermore, there are higher cases of tuberculosis (TB) in Ethiopia due to poverty, overcrowding, large families, and subpar living conditions.

Children who were classified as anemic had a 3.83 times higher risk of getting TB infections than non-anemic children. Supported by Northern Ethiopia [34]and Benshangul Gumuz [36] , this is because anemia highly prevalent in TB cases due to persistent inflammation or chronic diseases[45], and anemia appears to have predictive value for incident TB disease during ART [46].

Likewise, the risk of developing TB for children who were not getting TPT was 3.78 times higher as compared with those who were taking TPT at baseline. This finding is supported by different studies conducted in Ethiopia [34, 47], Tanzania [48], and global data[49]. TPT decreases mycobacterium load and reduces the progression of latent TB bacilli to active TB. Increases in mycobacterial load were associated with progressive impairment of mycobacterium specific-T-cell responses and increased the occurrence of active TB [50]. Additionally, the synergetic effect of ART and TPT in reducing active TB incidence is increased as a result of CD4+ T cell recovery

and viral load suppression [25]. Lower TPT intake is associated with an increased incidence of tuberculosis; in our study, only 63.42% of children with HIV took TPT.

The hazard of TB among children with wasting at baseline was 2.53 times more likely as compared to their counterparts. This finding is supported by studies conducted in Northern Ethiopia, Debre Markos [32, 34], and Southern Ethiopia [51]. This could be explained by the fact that malnutrition causes nutritionally acquired immune dysfunction and increases host susceptibility to infections. Under-nutrition also weakens the immune system through atrophy of the thymus, spleen, and lymph nodes and reduces cell-mediated immunity [52, 53]. Immunological and clinical recovery are directly associated with nutritional status [54].

Furthermore, children with late ART initiation had a 3.27 times increased risk of developing TB when compared to those with rapid ART initiation. This finding was in agreement with those of a study done in Ethiopia [32]. Initiation of ART early after confirmation of HIV-infection, even at a higher CD4 cell count, helps to reduce the occurrence of TB. This might be due to the early initiation of ART to reduce delays and improve viral suppression rates in children with HIV, increase retention care, and reduce HIV transmission [55].

Strength and limitation of the study

As a strength, this study was multicenter, covering wide ranges in geographical areas of specialized hospitals, and used a longer follow-up period for better estimation of the incidence of tuberculosis. There are certain inherent limitations to the current study. Owing to financial limitations, factors such as housing characteristics, the environment, and family history of smoking-related factors were evaluated retrospectively rather than prospectively in order to determine the incidence and predictors of TB following the start of ART. Additionally, the study excluded children who began antiretroviral therapy (ART) at the outset and got tuberculosis within a month or less. This may have led to an underestimation of the incidence of tuberculosis.

Conclusion and Recommendation

In this study, the incidence of TB was found to be high. Anemia, wasting, TB preventive therapy, and early initiation of ART were factors found to be significant predictors of TB incidence in HIV-infected children receiving ART. Additionally, clinicians should emphasize early screening and maximizing nutritional supplements for HIV-infected children to decrease the incidence of TB in those individuals. Furthermore, it needs emphasis on early detection and treatment of anemia and

TB preventive therapy, counseling on nutritional improvement, and timely initiation of ART to avert TB.

Abbreviations

AHR: Adjusted Hazard Ratio; ART: Antiretroviral Therapy; CPT: Cotrimoxazole Preventive Therapy; CD4: Cluster of Differentiation 4; OIs: Opportunistic Infections; CYO: Child-Year-Observations; SD: Standard Deviation; TB: Tuberculosis; TPT: Tuberculosis Preventive Treatment; UoG: University of Gondar; WHO: World Health Organization; AIC: Akakie Information Criterion; BIC: Bayesian Information Criterion; PHA: proportional hazard assumption

Ethical approval and consent to participate

This study used secondary data or medical charts; the need for informed consent and ethical approval was waived by the Institutional Review Board of the University of Gondar, College of Medicine and Health Sciences, on behalf of the Ethical Review Committee of the School of Nursing (SN/212/2022). Finally, before collecting the data, a letter of permission to collect the data was obtained from each Comprehensive Specialized Hospital administrator to collect the data from each ART clinic. At the time of abstraction, personal identifiers (names and contact numbers) were excluded. The data were kept strictly confidential and used only for study purposes. All of the procedures were carried out by considering the Declaration of Helsinki.

Patient and public involvement

Patients and public were not directly involved in the design, conduct and dissemination of this study.

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Authors' contributions

GBM worked on developing the research idea, designing the study, being involved in proposal writing, training and supervising the data collectors, analyzing and interpreting the results, and

preparing the manuscript. BTL, FDB, and WTW played their role in critically revising the proposal, participating in its design, analyzing and interpreting the results, and writing the manuscript. All authors were involved in reading and approving the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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Supplementary table 1: Estimated sample size determination by predictor variables for incidence of tuberculosis among HIV-infected children on ART, by using STATA version 17, cox-model

Variables	AHR	Power	Probability of withdrawal	Probability of event	Sample size (n)
Height for Age Z score	2.14	80%	0.15	0.167	383
WHO clinical staging	3.23	80%	0.15	0.167	161
ART adherence	2.05	80%	0.15	0.167	430

Supplementary table 2: Baseline clinical, nutritional, and laboratory characteristics of incidence of Tuberculosis among HIV-infected children on ART in Amhara Regional State Comprehensive Specialized Hospitals, Ethiopia

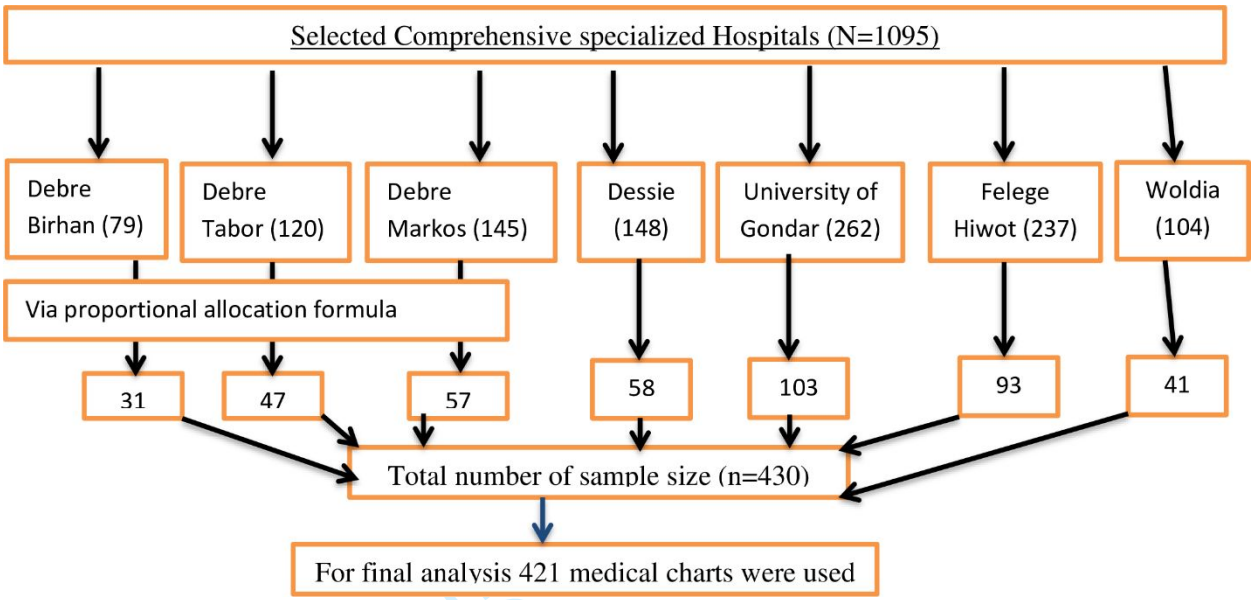
Variables	Categories	Frequency	Percent (%)
CD4 cell count			
	Above threshold	**336**	**79.81**
	Below threshold	**85**	**20.19**
Anemia			
	No	**348**	**82.66**
	Yes	**73**	**17.34**
Functional status (n=299)			
	Working	**201**	**67.22**
	Ambulatory	**95**	**31.77**
	Bedridden	**3**	**1.00**
Developmental status (n=122)			
	Appropriate	**82**	**67.21**
	Delayed	**35**	**28.69**
	Regressed	**5**	**4.1**
Wasting			
	No	**299**	**71.02**
	Yes	**122**	**28.98**
Stunting			
	No	**218**	**51.78**
	Yes	**203**	**48.22**
Previous Opportunistic infections			
	No	**323**	**76.72**
	Yes	**98**	**23.28**
WHO Clinical staging			
	Stage I	**261**	**62.00**
	Stage II	**84**	**19.95**
	Stage III	**55**	**13.06**
	Stage IV	**21**	**4.99**
ART drug adherence level			
	Good	**296**	**70.31**
	Fair	**68**	**16.15**
	Poor	**57**	**13.54**
Tuberculosis preventive Treatment (TPT) taken			
	Yes	**267**	**63.42**
	No	**154**	**36.58**
Cotrimoxazole preventive Therapy (CPT) taken			
	Yes	**345**	**81.95**
	No	**76**	**18.05**
ART side effect			
	No	**275**	**65.32**
	Yes	**146**	**34.68**
Presence regimen change			

Yes	**214**	**50.83**
No	**207**	**49.17**
Initiated ART within seven days		
Yes	**231**	**54.87**
No	**190**	**45.13**
DTG contained ART drugs		
Yes	**69**	**16.39**
No	**352**	**83.61**

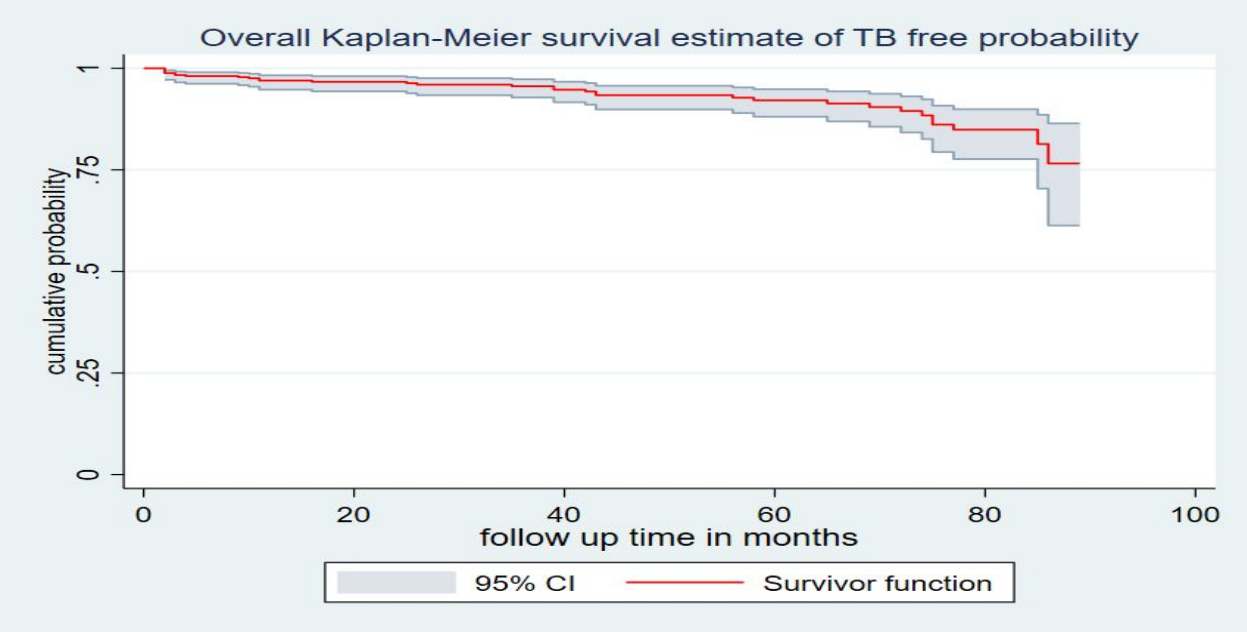
Supplementary table 3: Summary of model comparison between Semi parametric and parametric cox-regression model using log likelihood, AIC, and BIC criteria

Methods	Cox-model	Weibul	Exponential	Gompertz	Lognormal	Log-logistic
Log-likelihood	-168.36	-139.6	-139.62	**-138.43**	-141.27	-139.96
AIC	294.64	240.79	238.94	**236.02**	238.19	240.53
BIC	355.28	309.52	303.62	**304.74**	306.92	309.26

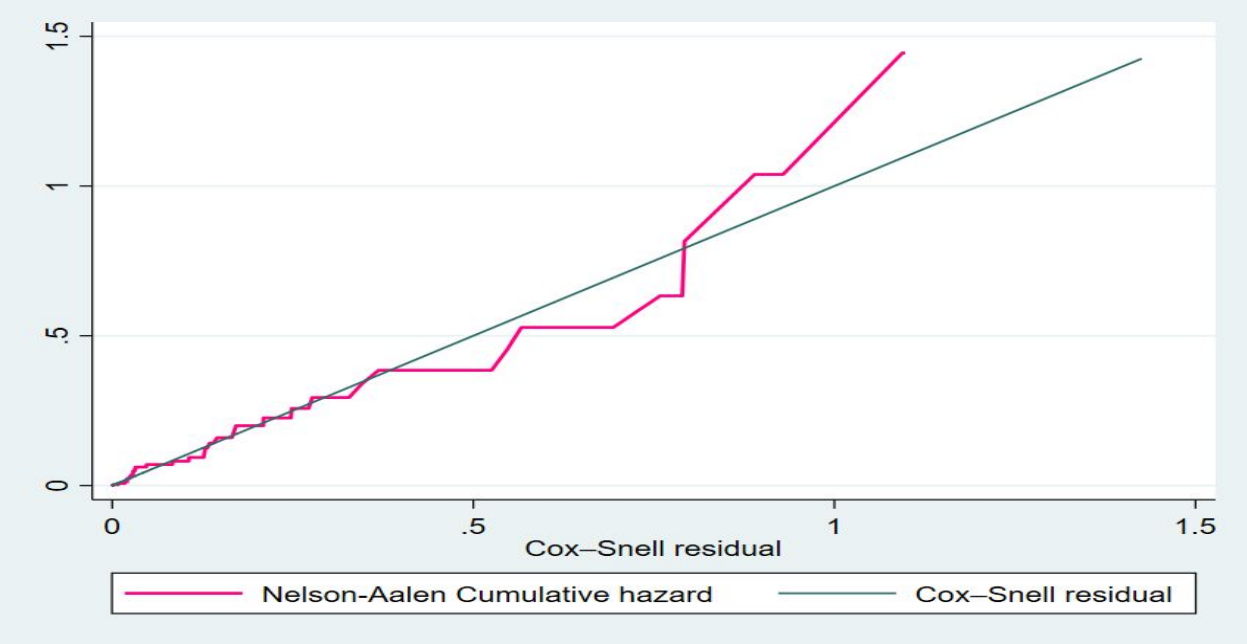
* Akakie Information Criterion (AIC), Bayesian Information Criterion (BIC)



Supplementary figure 1: schematic diagram of the sampling procedure for the incidence and predictors of Tuberculosis among HIV-infected children on ART in Amhara Region Comprehensive Specialized Hospitals, Ethiopia



Supplementary figure 2: overall Kaplan-Meier estimate of TB-free probability in children on ART in Amhara Region comprehensive specialized Hospitals



Supplementary figure 3: The goodness of fit test for the Gompertz regression model for the incidence of TB among children on ART in Amhara region comprehensive specialized Hospitals

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Incidence of Tuberculosis and its predictors among children on antiretroviral therapy in Amhara region, Ethiopia: A multicentre institution-based retrospective follow-up study

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Abstract:

Introduction: Tuberculosis (TB) continues to be a significant public health issue, particularly in low- and middle-income countries. Globally, the end-TB strategy targets an 80% reduction in TB incidence by 2030. Despite this strategy, there is limited evidence on the incidence of TB among HIV-infected children in the study area after the test and treatment strategies in the Amhara region. Hence, this study aimed to assess the incidence of TB and its predictors among children on antiretroviral therapy (ART) in the Amhara region, which is the second largest region in Ethiopia and located in the Northwestern, Northeastern, and North-central parts of Ethiopia.

Methods: A multicentre institution-based retrospective follow-up study was conducted on 421 HIV-infected children receiving ART from July 2014 to March 2022. The study participants were selected using a simple random technique. National antiretroviral intake and follow-up forms were utilized to gather data through the KoBo Toolbox. STATA version 17 was used for data analysis. The Kaplan-Meier curve was applied to estimate failure time, and the log-rank test was employed to compare groups of predictors. To find TB predictors, Gompertz regression models, both bivariable and multivariable, were constructed. Ultimately, a 95% confidence interval-adjusted hazard ratio was calculated, and variables with a p-value less than 0.05 were considered statistically significant.

Results: A total of 421 children with a record completeness rate of 97.9% were analyzed in the study. The tuberculosis incidence rate in children on ART was 2.16 (95% CI: 1.52, 3.05) per 100 child-year observations. Those children who were on baseline anemic [AHR: 3.83 (95% CI: 1.46, 10.04)], never took TB preventive treatment (TPT) [AHR: 3.78 (95% CI: 1.44, 9.94)], wasted children [AHR: 2.53 (95% CI: 1.19, 5.38)], and not initiated antiretroviral therapy within seven days [AHR: 2.35 (95% CI: 1.15, 4.78)] were the significant predictors.

Conclusion: The incidence of TB in children on ART was relatively high. HIV-positive children presenting with anaemia, who never took TPT, wasted, and late initiation of ART were prone to the occurrence of TB. Therefore, prioritizing anaemia treatment, TB preventive therapy, nutritional counseling, and timely ART initiation is essential to curb the TB burden.

Keywords: Antiretroviral therapy; HIV-infected children; incidence; predictors; test and treat, Tuberculosis

Strength and limitations of the study

- As a strength, this study was multicentre, covering wide ranges in geographical areas of specialized comprehensive hospitals
- Due to the nature of retrospective follow-up study, factors such as housing characteristics, the environment, and family history of smoking-related factors were not evaluated
- The study excluded children who started ART and developed tuberculosis within a month, potentially underestimating tuberculosis incidence
- Since the study was conducted in tertiary level of health care (comprehensive specialized hospitals) subjected to biases related to healthcare accessibility

Introduction

TB remains an important public health problem, particularly in low-and middle-income countries (LMIC) [1]. Globally, TB incidence in 30 high-burden countries accounts for 87% of cases [2]. Children and young adolescents (aged below 15 years) account for 1.25 million (12%) of the total TB incidence across the globe every year [3].

The risk of developing this global infectious disease is 18 times higher among HIV-infected individuals [4]. It remains the second leading cause of death from infectious disease, and the risk of death is threefold higher among HIV-infected individuals worldwide [1, 4]. Recently, the global occurrence of TB mortality in children increased significantly, which was 214,000 in 2023. Each day, 600 children lost their lives to this preventable disease. Approximately 31,000 deaths are related to people with HIV [2, 5].

In Sub-Saharan African countries, the HIV-TB burden has increased, despite the test-and-treat policy in this region. The incidence rate of tuberculosis among HIV-infected children is 3.42 per 100-person year [6]. This problem has a significant economic burden in low and middle- income countries [1]. Similar to other Sub-Saharan countries, tuberculosis remains a major health issue among HIV-infected children in Ethiopia, with an incidence rate of 4.33 cases per 100 person-years [7].

The occurrence of tuberculosis (TB) among children on ART treatment is 24.2% to 32% in Nigeria [8-10] and 15.2% in Ethiopia [11], while the incidence of TB among children with HIV varies from 0.83 to 2.3 in China [12], 7 per 1000 person-years in Thailand [13], 4 in South Africa [14], 2 to 2.63 in Ethiopia [15-17], and 17.4 in East Africa [18].

Some studies showed that severe immunosuppression, age less than two years, WHO clinical staging [19-21], underweight [21], a low Cluster of Differentiation 4 (CD4) cell count, anaemia [22], and virological failure [23] contribute to the occurrence of TB among children on ART treatment. To better understand the variety of contexts and the application of new approaches in factors that affect the risk of TB in children living with HIV, additional investigation is necessary.

The achievement of Ethiopia's Sustainable Development Goals (SDGs) and the end-TB strategy targets established by the World Health Organization (WHO) and the United Nations, which aim

to reduce tuberculosis cases by 80% and deaths from tuberculosis by 90%, provide support for this study [3, 24]. The political declaration of the UN high-level meeting on TB held in WHO plans to reach TB Preventive Treatment (TPT) at least 90% of people with HIV in 2027 [25].

Ethiopia has adopted a test-and-treat strategy since June 10, 2014, for all children initiating ART regardless of CD4 cell count and WHO clinical staging, aiming at the reduction of HIV-related mortality and morbidity [26].

Despite this intervention, there is a paucity of evidence of the overall incidence of TB in children enrolled in the Amhara region after the test and treat strategy. In addition to previous studies, this finding covers a wide range of public institutions, and in this study, rapid initiation of ART and Dolutegravir (DTG)-contained ART drugs were incorporated as independent variables to identify the incidence of TB. The current study population also consists of newly enrolled pediatric HIV care patients, rather than ever enrolled on antiretroviral therapy. Timely and recent evidence is very crucial. Furthermore, more research is required to incorporate these variables and new strategies. The findings will help decision-makers and policymakers at different levels with HIV/AIDS care and assistance.

Therefore, this study aimed to assess the incidence of TB and its predictors among HIV-infected children enrolled in ART in the Amhara Region.

Methods

Study Design, setting, and period

A multicentre institution-based retrospective follow-up study was conducted among HIV-positive children enrolled at Amhara region comprehensive specialized hospitals from July 2014 to March 2022. In Ethiopia, Comprehensive Specialized Hospitals represent the highest level of healthcare facilities (Tertiary level of health services) which provides advanced medical services, specialized care, and training centre for health professionals.

The Amhara region is located in the Northwestern, Northeastern, and North-central parts of Ethiopia, with an estimated area of 159,173.66 square kilometers. The most recent estimated population in this region is 30,848,988 [27]. In this region, there are 81 hospitals; among those,

eight are comprehensive specialized and teaching hospitals: the University of Gondar, Tibebe-Ghion, Felege Hiwot, Debre Markos, Debre Tabor, Dessie, Woldia, and Debre Birhan.

Those hospitals have been providing ART care and support as part of the National AIDS Control Program. Among the total number of Comprehensive Specialized Hospitals, seven were included in the study area, except Tibebe-Ghion, due to an insufficient number of study participants. A total of 1095 children were newly enrolled in HIV care in the Amhara region from July, 2014 to March, 2022.

Population and eligibility

The source population for this study comprised all HIV-infected children who were enrolled in the pediatric HIV care clinics at comprehensive specialized hospitals in the Amhara Region and had a minimum of one month of ART follow-up. HIV-positive children who were newly enrolled in the pediatric HIV care clinic in Amhara Region from July, 2014 to March, 2022 were the study population. Children with HIV who already had TB before starting ART and records with incomplete baseline information (CD4 count, WHO staging, haemoglobin level, weight, and height) and unknown dates of ART initiation and TB occurrence were excluded from the study. **(Supplementary Figure 1)**

Sample size determination

The sample size was determined based on key predictors, including height for age, WHO clinical staging, and ART adherence level. Using STATA version 17 and the Cox model, the calculation was performed under the following assumptions, a critical value ($Z\alpha/2$) of 1.96 for a 5% significance level, a critical value ($Z\beta/2$) of 0.84 for 20% power, an event probability of 0.167 [28], and the probability of withdrawal was 0.15 using an adjusted hazard ratio (AHR). (T**able 1)**

Table 1: Estimated sample size determination by predictor variables for HIV-infected children on ART, by using STATA version 17, cox-model

Variables	Hazard Ratio	Power	Probability of withdrawal	Probability of event	Sample size (n)
Height for Age Z score	2.14	80%	0.15	0.167	383

WHO clinical staging	3.23	80%	0.15	0.167	161
ART adherence	2.05	80%	0.15	0.167	430

Sampling procedures, and sampling technique

From the calculated sample sizes, the largest sample size (430) was selected as a sample for this study. The sample was allocated proportionally to those comprehensively specialized hospitals in the Amhara region, and records were selected using simple random techniques. **(Supplementary Figure 2)**

Variables of the study

The outcome variable for this study was the occurrence of TB during the follow-up period. The independent variables were socio-demographic characteristics: age, sex, residence, current parent's status, educational status of the caregiver, HIV disclosure status, and marital status of the caregiver. Baseline clinical, nutritional, and laboratory characteristics: CD4 count, WHO clinical staging, haemoglobin level, anthropometric indices, previous OIs, functional and developmental status. ART and medication-related characteristics: baseline ART regimen, DTG containing ART drugs, presence of regimen change, level of ART adherence, TPT, CPT, ART side effects, initiation of ART.

Operational definition of the variables

Events: The occurrence of TB among HIV-infected children during the follow-up period at any time after enrollment in the pediatric HIV care clinic, as confirmed by a health care professional.

Tuberculosis (TB) is defined as a diagnosis of either pulmonary tuberculosis (PTB) or extra-pulmonary tuberculosis (EPTB) as documented in children's medical records during the study period.

Censored: Children, who were lost to follow-up, dropped out, transferred out, died due to any causes, or completed the study period before developing TB**.**

Pulmonary Tuberculosis (PTB): was diagnosed based on the national TB diagnosis guideline, which primarily affects the lungs, and diagnosis is based on clinical symptoms, chest X-ray findings, sputum smear microscopy, or molecular tests such as GeneXpert, or histopathology.

Extra-pulmonary Tuberculosis (EPTB): Defined as TB that affects organs outside the lungs, such as lymph nodes, pleura, bones and joints, meninges, or the abdomen. Diagnosis may involve biopsy, imaging, or fluid analysis.

Wasting: if the weight for height (WFH) Z-score is less than -2 SD for less than five years, or if the body mass index (BMI) for age Z-score is less than -2 SD for greater than five years [29].

ART adherence levels: categorized as follows: good (≥95% compliance or ≤3 missed doses per month), fair (85–94% compliance or 4–8 missed doses per month), and poor (<85% compliance or ≥9 missed doses per month), as recorded by ART health personnel [29] .

Rapid initiation of ART refers to starting antiretroviral therapy on the same day as HIV diagnosis or within seven days afterward [30].

Urban areas are officially designated towns with municipal structures and better access to services like education, healthcare, electricity, and water[31].

Rural areas are primarily agricultural or pastoral, with limited infrastructure such as roads, electricity, healthcare, and schools, and characterized by dispersed settlements[31].

Anaemia was defined as having a haemoglobin level of less than 10 mg/dl [32].

Data collection tool and procedures

Data were retrospectively collected using a data extraction tool based on Ethiopian ART guidelines. Information was obtained from ART intake form, follow-up form, and children's medical charts for patients newly enrolled at ART clinics from July 2014 to March 2022 [29]. Data was extracted for one month, from May 17 to June 15, 2022. The variables include socio-demographic, clinical, laboratory, ART-related, and medication-related factors. Data collection was conducted by seven bachelor's degree nurses who had smartphones and prior experience with ART follow-up, as well as basic training in ART management.

Data quality control

The data extraction tool was pretested on 5% of the sample size at the University of Gondar Comprehensive Specialized Hospital prior to the actual data collection. Additionally, one-day onsite training was conducted for data collectors and supervisors, covering topics such as reviewing ART follow-up and medical records, data collection techniques, and the study's objectives. Data collection was carried out using the KoBo toolbox, which was prepared with relevant restrictions by trained nurses working in Hospitals. Besides, the data collector and

principal investigators carefully monitored the entire data collection process and daily submission report.

Data processing and analysis

The KoBo toolbox [33] was used for data collection, then exported and imported into STATA version 17 statistical software [34] for final analysis. Additionally anthropometric indices were created using the WHO anthro and WHO anthroPlus software. To describe the data, descriptive statistics including mean (±SD), percentage, and frequency were employed. The Variance inflation factor (VIF) was used to check the association between predictor variables; the average VIF was found to be 1.18, which indicates no significant multicollinearity**.**

A separate graph of Kaplan-Meier survival functions and the log-rank test were estimated for each categorical variable to compare survival between different exposure groups. The proportional hazard assumption (PHA) was checked using both graphical and statistical global tests and revealed that the PHA was satisfied. The log-likelihood and Akaike Information Criteria (AIC) were applied to select the best-fitted model, and a model with a minimum AIC was considered the best-fitted model.

In our survival analysis, we consider parametric models including the Exponential, Weibull, Log-normal, Log-logistic, and Gompertz regression models. Models were evaluated based on the Akaike Information Criterion (AIC) to identify the best fit. The Gompertz model, with the lowest AIC value of 236.02, was selected as the best-fitted model. This indicates that among the models considered, the Gompertz model provided the best balance between model fit and complexity.

In addition, the goodness-of-model fitness was also checked using the Cox-Snell residual test. Variables having a p-value less than 0.25 in the Bivariable analysis were fitted into the multivariable Gompertz regression model. A hazard Ratio with a 95% CI was used to determine the strength of the association. Variables with a $p<0.05$ in multivariable analysis were considered statistically significant.

To handle and manage missing data, multiple imputations using multivariable chained equations (MVCE) were carried out to handle and manage missing data. The variables missing at random (MAR) are clarified by the "Little test" results for continuous variables and graphical patterns for

categorical variables, making the application of multiple imputations straightforward. Furthermore, sensitivity analysis was performed utilizing both descriptive and inferential statistical techniques to confirm if a significant difference was detected between the outputs of the original and imputed data.

Patient and public involvement

Patients and the public were not directly involved in the design, conduct and dissemination of this study.

Results:

1. Socio-Demographic characteristics

A total of 430 medical records of HIV-infected children on ART were reviewed. Of these, 421 (97.9%) were included in this study, and the remaining percent were excluded from the analysis due to data incompleteness. The mean (SD) age of the study participant was 7.58 (±4.02) years; one-third was found in the age group of 5-9 years. Nearly two-thirds (58.43%) were male, and 70% were from urban areas. **(Table 2)**

Table 2: Socio-demographic characteristics of HIV-infected children on ART in Amhara Region comprehensive specialized hospitals, Ethiopia, 2022 (n=421)

Variables	Categories	Frequency(n)	Percentage (%)
Age			
	Less than 5 Years	122	28.98
	5-9 Years	145	34.44
	10-14 Years	154	36.58
Sex			
	Female	175	41.57
	Male	246	58.43
Residence			
	Rural	126	29.93
	Urban	295	70.03
Current parent status			
	Both parents alive	290	68.88
	One parent alive	113	26.84
	Both parents deceased	18	4.28
Educational Status of the caregiver			
	No formal education	144	34.2
	Primary	150	35.63
	Secondary	67	15.91

Tertiary (College & above)	60	14.25
Marital status of the caregiver		
Married	228	54.16
Unmarried	87	20.67
Divorced	70	16.63
Widowed	36	8.55
HIV status of the parents		
Non-reactive	158	37.53
Reactive	263	62.47
Disclosure status the child		
Yes	220	52.26
No	201	47.74

2. Baseline clinical, laboratory, nutritional and ART medication related characteristics

At baseline, 79.9% of children were in WHO clinical stages I or II. The median CD4+ count was 421 (IQR: 403, 1017) cells/l, and one-fifth (20.19%) had CD4+ below the threshold level. The median haemoglobin level was 12.3 (IQR: 10.7, 13.6) mg/dl, and 17.34% had less than 10 mg/dl haemoglobin level. Nearly 30% and 50% of children were wasted and stunted at baseline, respectively. Out of 421 HIV-infected children, 296 (70.31%) had a good ART drug adherence level. More than two-thirds (63.42%) of children had never received TPT, and 45.13% of children had never taken ART within seven days of its duration. **(Supplementary table 1)**

3. Follow-up time and incidence of TB incidence

A total of 421 children followed from 1 to 89 months. The mean follow-up time was 42 months (14.57 to 70.07 months). The total time at risk was 1484.75 child-year observations (CYO). New TB cases were observed in 32 (7.6%) children; the overall TB incidence rate was 2.16 (95% CI: 1.52, 3.05) per 100 CYO. **(Figure 1)**

The incidence rate of TB was 3.98 (95% CI, 1.99, 7.95) per 100 CYO in the six months of ART initiation and decreased to 0.33 (95% CI, 0.05, 2.34) per 100 CYO after three years of ART initiation. The cumulative probability of TB incidence at the end of two, four, and six years of the study was 0.9636, 0.9309, and 0.9045, respectively. The incidence rate of TB was 8.09 (95% CI, 5.03, 13.01) per 100 CYO among anemic children, while it was 1.18 (95% CI, 0.7, 1.95) per 100 CYO among non-anemic children. **(Supplementary Figure 3, 4, 5, and 6)**

4. Model comparison

The proportional hazard assumption, semi-parametric, and parametric proportional hazard models were fitted to estimate the incidence of tuberculosis and its predictors among HIV-infected children in the Amhara region. The graphical test and global test (P-value = 0.2980) confirmed the proportional hazard assumption (PHA). The most fitted model was chosen using Akaike information criteria (AIC = 236.02), Bayesian information criteria (BIC = 304.74), and log-likelihood (-138.43). The results outperformed the Cox-proportional hazard and other parametric models in all three comparison techniques. As a result, interpretations and conclusions were based on the Gompertz model. **(Supplementary Table 2)**

The Cox-Snell residual test checked the goodness of fit. **(Figure 2)** The shared frailty was estimated, (theta: $1.89e^{-07}$), and the distribution of unmeasured variables is indifferent between the comprehensive specialized hospitals.

5. Predictors of incidence of Tuberculosis

In bi-variable parametric survival Gompertz regression: parental status of the caregivers, educational status of the caregivers, baseline of WHO clinical staging, CD4+ count, haemoglobin status, functional and developmental status, regimen change, ever taking TPT, ever taking CPT, ART adherence level, previous opportunistic infections, dolutegravir (DTG)-contained ART drugs, wasting, and rapid initiation of ART were found to be significant predictors of TB among children in ART. In the final multivariable Gompertz regression model, only baseline haemoglobin, ever taking TPT, wasting, and rapid initiation of ART significantly increased the incidence of tuberculosis.

Anaemic children had 3.83 times [AHR: 3.83 (95% CI: 1.46, 10.04)] higher risk of getting TB infections than those in non-anemic children. Likewise, the risk of developing TB for children who were not given TPT was 3.78 times [AHR: 3.78 (95% CI: 1.44, 9.94)] higher as compared with those who were given TPT at baseline. Additionally, the hazard of TB among children with wasted children at baseline was 2.53 times [AHR: 2.53 (95% CI: 1.19, 5.38)] more likely as compared to their well-nourished counterparts. Furthermore, children had late ART initiation 3.27 times [AHR: 3.27 (95% CI: 1.26, 8.46)] increased risk of developing TB compared to rapid initiation of ART **(Table 3).**

Table 3: Bi-variable and multivariable Gompertz Cox-regression analysis of predictors of Tuberculosis among children on ART in Amhara Region Comprehensive Specialized Hospitals, Ethiopia, 2022 (n = 421).

Variables	Status		CHR (95% CI)	AHR (95% CI)	P value
	Event (n=32)	Censored (n=389)			
Parental status of the child					
Both parents alive	19	271	**1**	**1**	
One or both parents dead	13	118	1.77 (1.88, 3.59)	0.8 (0.32, 2.02)	0.64
Educational status of the caregiver					
No formal education	9	135	1.27(0.49, 3.29)	0.72(0.25, 2.04)	0.53
Primary	15	117	2.05(0.87, 4.83)	0.97(0.33, 2.81)	0.96
Secondary and Tertiary	8	137	1	1	
WHO Clinical staging					
Stage I & II	17	328	**1**	**1**	
Stage III & IV	15	61	5.47 (2.73, 11.00)	1.71(0.62, 4.71)	0.299
CD4 cell count					
Above threshold	21	315	**1**	**1**	
Below threshold	11	74	2.89(1.39, 6.02)	1.59(0.59, 4.31)	0.357
Anaemia					
No	15	333	**1**	**1**	
Yes	17	56	7.26(3.62, 14.56)	3.83 (1.46, 10.04)	0.006
Working and appropriate motor developmental status					
Yes	16	267	**1**	**1**	
No	16	122	2.39(1.19, 4.8)	1.66(0.77, 3.61)	0.196
Adherence level of ART					
Optimal	16	280	**1**	**1**	
Sub-optimal	16	109	2.79(1.39, 5.59)	0.92(0.36, 2.4)	0.874
ART regimen change					
Yes	15	199	**1**	**1**	
No	17	190	1.63(0.81, 3.27)	0.77 (0.32, 1.87)	0.57
Ever taking tuberculosis preventive treatment(TPT)					
Yes	7	260	**1**	**1**	
No	25	129	7.55(3.26, 17.47)	3.78(1.44, 9.94)	0.007
Ever taking Cotrimoxazole preventive therapy(CPT)					
Yes	21	324	**1**	**1**	
No	11	76	2.68(1.29, 5.57)	1.28(0.5, 3.27)	0.601
Weight for height					
No wasting	15	284	**1**	**1**	
Wasting	**17**	**105**	**2.9(1.45, 5.81)**	**2.53(1.19, 5.38)**	**0.016**
Previous opportunistic infections					
No	17	306	**1**	**1**	
Yes	15	83	3.43(1.71, 6.88)	1.32 (0.54, 3.19)	0.54

Doultegravir (DTG) contained drugs					
Yes	6	63	**1**	**1**	
No	26	326	0.46 (0.18, 1.14)	0.37 (0.13, 1.03)	0.059
Initiation of Antiretroviral therapy					
Rapid Initiation	6	**225**			
Late Initiation	**26**	**164**	**4.41(1.81, 10.74)**	**3.27(1.26, 8.46)**	**0.015**

**** Significant at α 0.05, 1: reference, *CHR, crude hazard ratio, AHR, Adjusted hazard ratio***

Discussion

This study aimed to assess the TB incidence rate and its predictors in children on ART in Amhara region comprehensive specialized Hospitals.

In this study, HIV-infected children on ART were 2.16 (95% CI 1.52, 3.05) per 100 child-years observations(CYO), which is consistent with study conducted in Debre Markos 2.63 [35], in Northeast Ethiopia 2.0 [36], and Southern Ethiopia 2.6 per 100 CYO[37]. This finding is lower than study conducted in Northern Ethiopia [38], in Benishangul Gumuz region in Northwest Ethiopia [39, 40], in Adama Referral Hospital and Medical College [41], in Southwest Ethiopia [42], and in Tanzania [43]. This could be due to higher burden of tuberculosis in resource-limited settings[44], and also might be due to the difference in sociodemographic and baseline clinical characteristics of the study participant, changes in the study period and setting.

However, our study is higher than studies conducted in New York City [45], in Ireland and the United Kingdom [46], and in China [47]. This could be because the general population in high-income countries has a low incidence rate of tuberculosis. In addition, the use of early TB diagnosis and prevention techniques, along with the accessibility of modern technologies, significantly lowers the incidence of tuberculosis [48]. Furthermore, there are higher cases of tuberculosis (TB) in Ethiopia due to poverty, overcrowding, large families, and subpar living conditions.

Children who were classified as anemic had a 3.83 times higher risk of getting TB infections than non-anemic children. Supported by Northern Ethiopia [38]and Benshangul Gumuz [40] , this is because anaemia highly prevalent in TB cases due to persistent inflammation or chronic diseases[49], and anaemia appears to have predictive value for incident TB disease during ART [50].

Likewise, the risk of developing TB for children who were not getting TPT was 3.78 times higher as compared with those who were taking TPT at baseline. This finding is supported by different studies conducted in Ethiopia [38, 51], Tanzania [52], and global data[53]. TPT decreases mycobacterium load and reduces the progression of latent TB bacilli to active TB. Increases in mycobacterial load were associated with progressive impairment of mycobacterium specific T-cell responses and increased the occurrence of active TB [54].

Additionally, the synergetic effect of ART and TPT in reducing active TB incidence is increased as a result of CD4+ T cell recovery and viral load suppression [25]. Lower TPT intake is associated with an increased incidence of tuberculosis; in our study, only 63.42% of children with HIV took TPT.

The hazard of TB among children with wasting at baseline was 2.53 times more likely as compared to their counterparts. This finding is supported by studies conducted in Northern Ethiopia, Debre Markos [35, 38], and Southern Ethiopia [55]. This could be explained by the fact that malnutrition causes nutritionally acquired immune dysfunction and increases host susceptibility to infections.

Under-nutrition also weakens the immune system through atrophy of the thymus, spleen, and lymph nodes and reduces cell-mediated immunity [56, 57]. Immunological and clinical recovery are directly associated with nutritional status [58].

Furthermore, children with late ART initiation had a 3.27 times increased risk of developing TB when compared to those with rapid ART initiation. This finding was in agreement with those of a study done in Ethiopia [35]. Initiation of ART early after confirmation of HIV-infection, even at a higher CD4 cell count, helps to reduce the occurrence of TB. This could be attributed to the early initiation of ART, which helps minimize delays, enhances viral suppression rates in children with HIV, improves retention in care, and reduces the risk of HIV transmission [59].

This study was conducted in a tertiary-level, comprehensive specialized hospitals, which may introduce biases related to healthcare accessibility, particularly in LMIC with significant geographic and socioeconomic disparities. These hospitals primarily serve urban populations and provide advanced medical services, potentially limiting the generalizability of findings to

children in rural areas. A substantial proportion of ART children in the region, especially those with limited access to healthcare, were not represented.

Strength and limitations of the study

As a strength, this study was multicentre, covering wide ranges in geographical areas of specialized hospitals, and used a longer follow-up period for better estimation of the incidence of tuberculosis. There are certain inherent limitations to the current study. Owing to financial limitations, factors such as housing characteristics, the environment, and family history of smoking-related factors were evaluated retrospectively rather than prospectively in order to determine the incidence and predictors of TB following the start of ART. Additionally, the study excluded children who began antiretroviral therapy (ART) at the outset and got tuberculosis within a month or less. This may have led to an underestimation of the incidence of tuberculosis.

Conclusion and Recommendation

In this study, the incidence of TB was found to be high. Anaemia, wasting, TB preventive therapy, and early initiation of ART were factors found to be significant predictors of TB incidence in HIV-infected children receiving ART. Additionally, clinicians should emphasize early screening and maximizing nutritional supplements for HIV-infected children to decrease the incidence of TB in those individuals. Furthermore, it needs emphasis on early detection and treatment of anaemia and TB preventive therapy, counseling on nutritional improvement, and timely initiation of ART to avert TB. Future studies should include data from community-level health facilities to ensure more representative and equitable findings.

Abbreviations

AHR: Adjusted Hazard Ratio; ART: Antiretroviral Therapy; CPT: Cotrimoxazole Preventive Therapy; CD4: Cluster of Differentiation 4; OIs: Opportunistic Infections; CYO: Child-Year-Observations; SD: Standard Deviation; TB: Tuberculosis; TPT: Tuberculosis Preventive Treatment; UoG: University of Gondar; WHO: World Health Organization; AIC: Akakie Information Criterion; BIC: Bayesian Information Criterion; PHA: proportional hazard assumption

Ethical approval and consent to participate

This study used secondary data or medical charts; the study was approved by the Institutional Review Board of the University of Gondar, College of Medicine and Health Sciences, on behalf of the Ethical Review Committee of the School of Nursing (SN/212/2022) with a waiver of informed consent. Finally, before collecting the data, a letter of permission to collect the data was obtained from each Comprehensive Specialized Hospital administrator to collect the data from each ART clinic. At the time of abstraction, personal identifiers (names and contact numbers) were excluded. The data were kept strictly confidential and used only for study purposes. All of the procedures were carried out by considering the Declaration of Helsinki.

Patient and public involvement

Patients and the public were not directly involved in the design, conduct and dissemination of this study.

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Authors' contributions

GBM worked on developing the research idea, designing the study, being involved in proposal writing, training and supervising the data collectors, analyzing and interpreting the results, and preparing the manuscript. BTL, FDB, and WTW played their role in critically revising the proposal, participating in its design, analyzing and interpreting the results, and writing the manuscript. All authors were involved in reading and approving the final manuscript. GBM is responsible for the overall content as the guarantor.

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Availability of data and materials

Data will be available upon reasonable request from the corresponding author.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Figure 1: Overall Kaplan-Meier estimate of TB-free probability in children on ART in Amhara Region comprehensive specialized Hospitals, Ethiopia

Figure 2: The goodness of fit test for the Gompertz regression model for the incidence of TB among children on ART in Amhara region comprehensive specialized Hospitals, Ethiopia

Supplementary Figure 1: Diagram for study population selection for HIV-infected children on ART in Amhara Region Comprehensive Specialized Hospitals, Ethiopia

Supplementary Figure 2: Schematic diagram of the sampling procedure for among HIV-infected children on ART in Amhara Region Comprehensive Specialized Hospitals, Ethiopia

Supplementary Figure 3: Kaplan-Meier TB-free survival of anemic status a main predictor variables among children on ART in Amhara region comprehensive Specialized Hospitals, Ethiopia

Supplementary Figure 4: Kaplan-Meier TB-free survival of tuberculosis preventive therapy a main predictor variables among children on ART in Amhara region comprehensive Specialized Hospitals, Ethiopia

Supplementary Figure 5: Kaplan-Meier TB-free survival of Nutritional status a main predictor variables among children on ART in Amhara region comprehensive Specialized Hospitals, Ethiopia

Supplementary Figure 6: Kaplan-Meier TB-free survival of Initiation of Antiretroviral therapy a main predictor variables among children on ART in Amhara region comprehensive Specialized Hospitals, Ethiopia

Supplementary Table 1: Baseline clinical, nutritional, and laboratory characteristics of HIV-infected children on ART in Amhara Regional State Comprehensive Specialized Hospitals, Ethiopia

Supplementary Table 2: Summary of model comparison between Semi parametric and parametric Cox-regression model using log-likelihood, AIC, and BIC criteria

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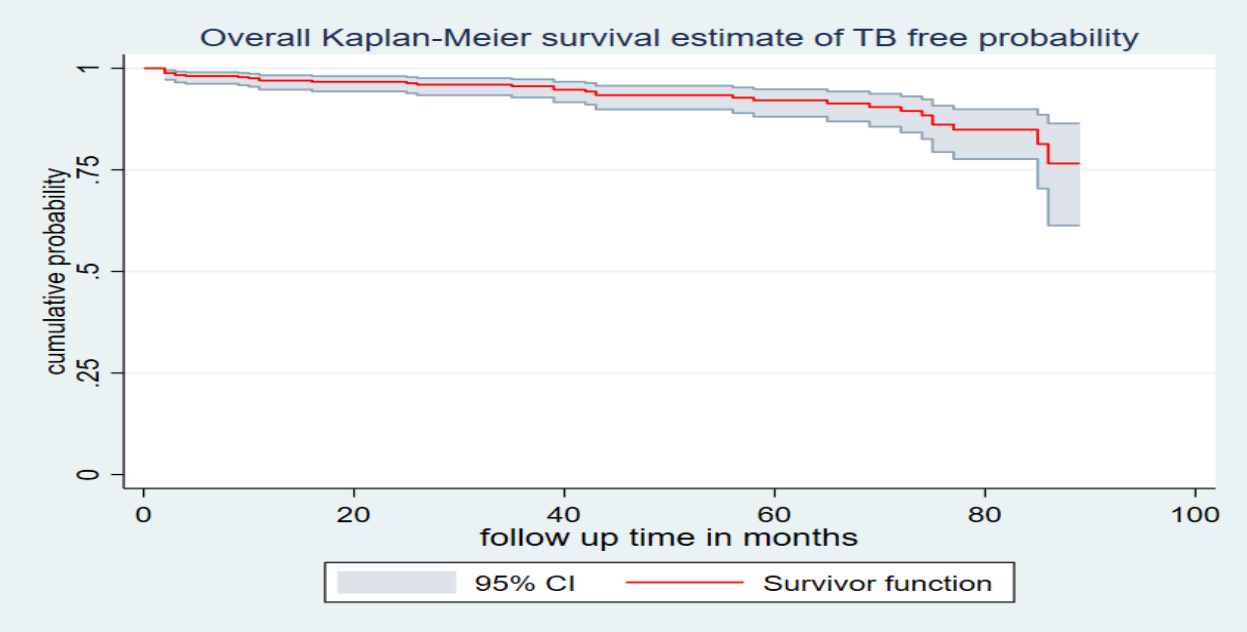


Figure 1: Overall Kaplan-Meier estimate of TB-free probability in children on ART in Amhara Region comprehensive specialized Hospitals, Ethiopia

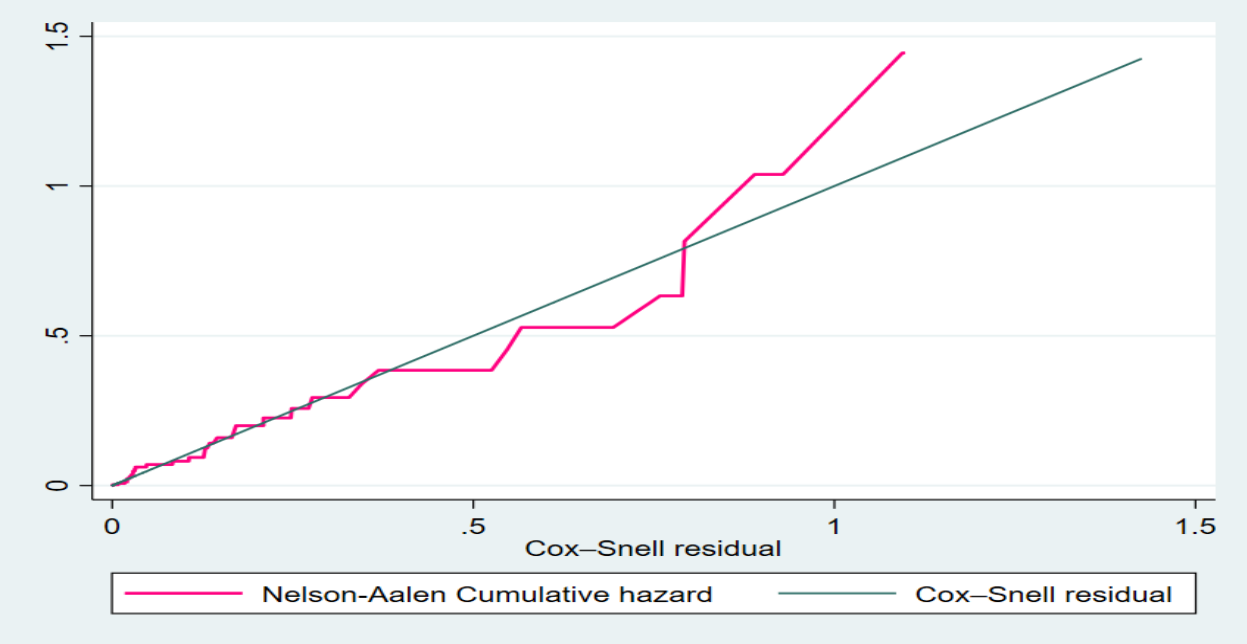
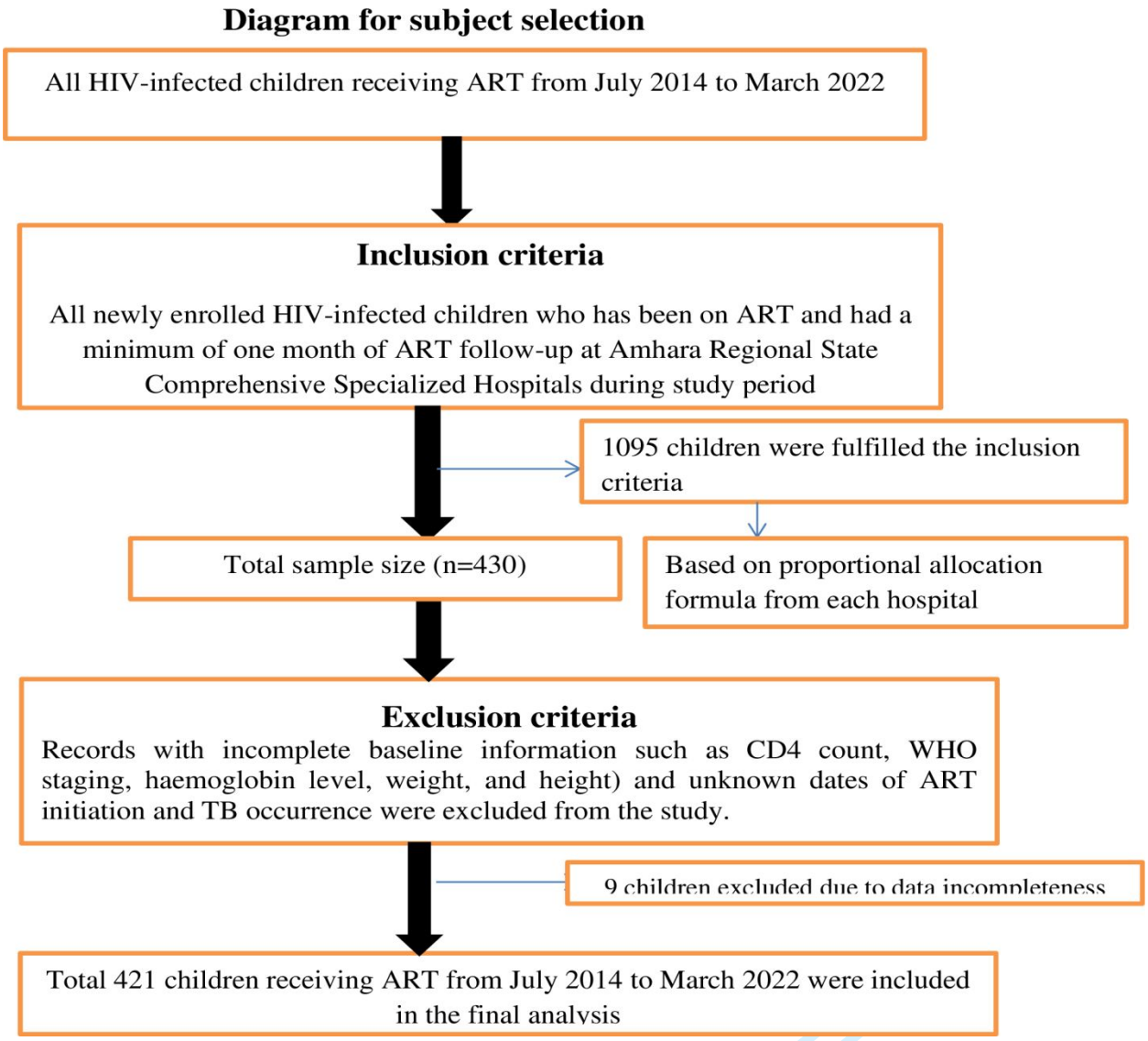
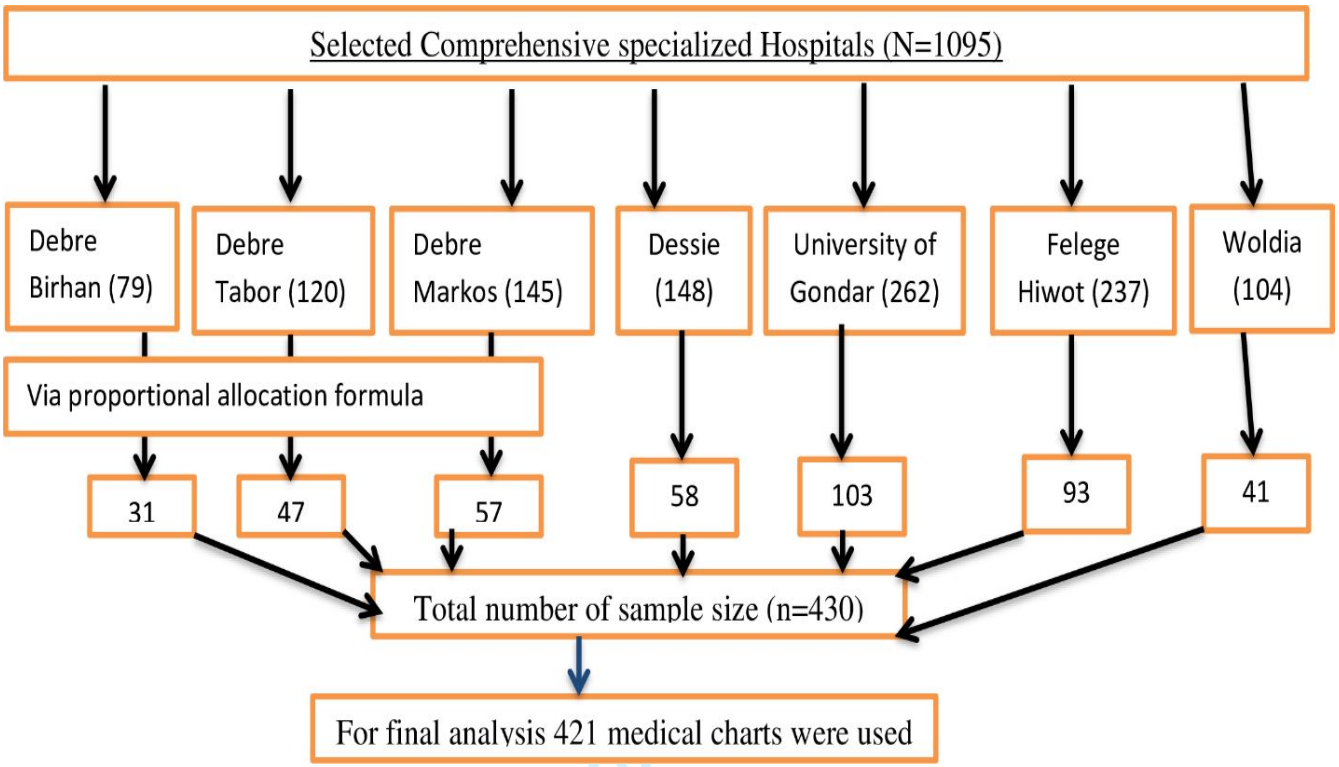


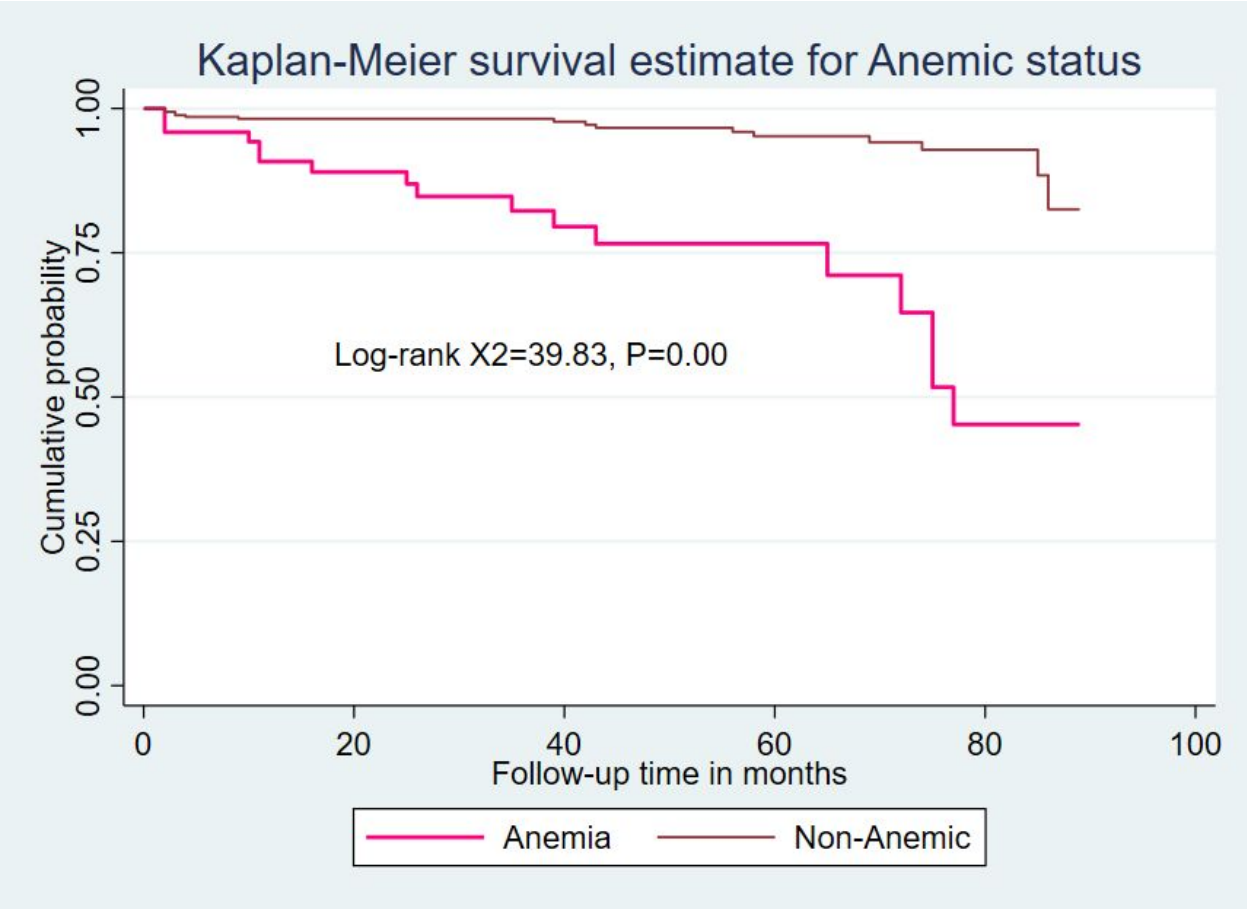
Figure 2: The goodness of fit test for the Gompertz regression model for the incidence of TB among children on ART in Amhara region comprehensive specialized Hospitals, Ethiopia



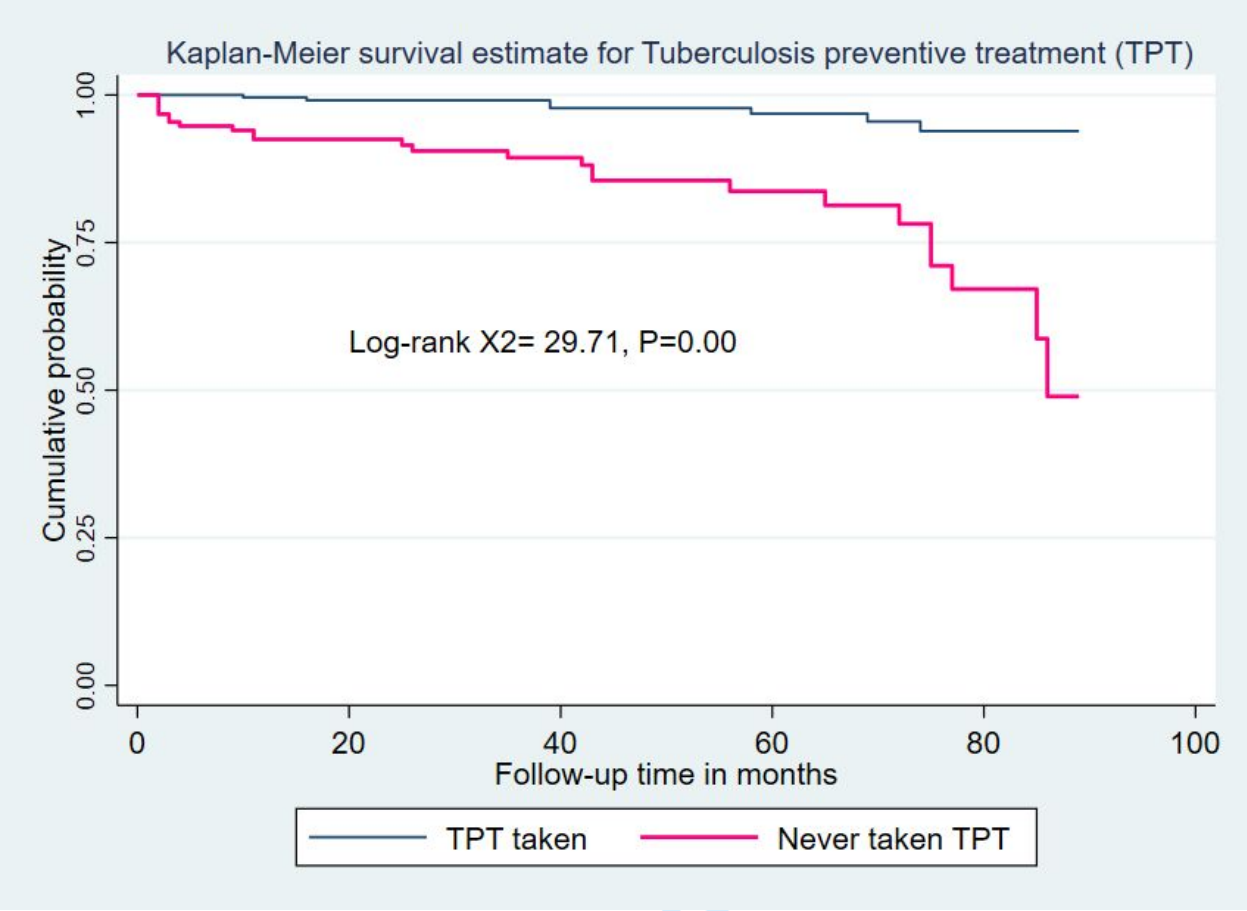
Supplementary Figure 1: Diagram for study population selection for HIV-infected children on ART in Amhara Region Comprehensive Specialized Hospitals, Ethiopia



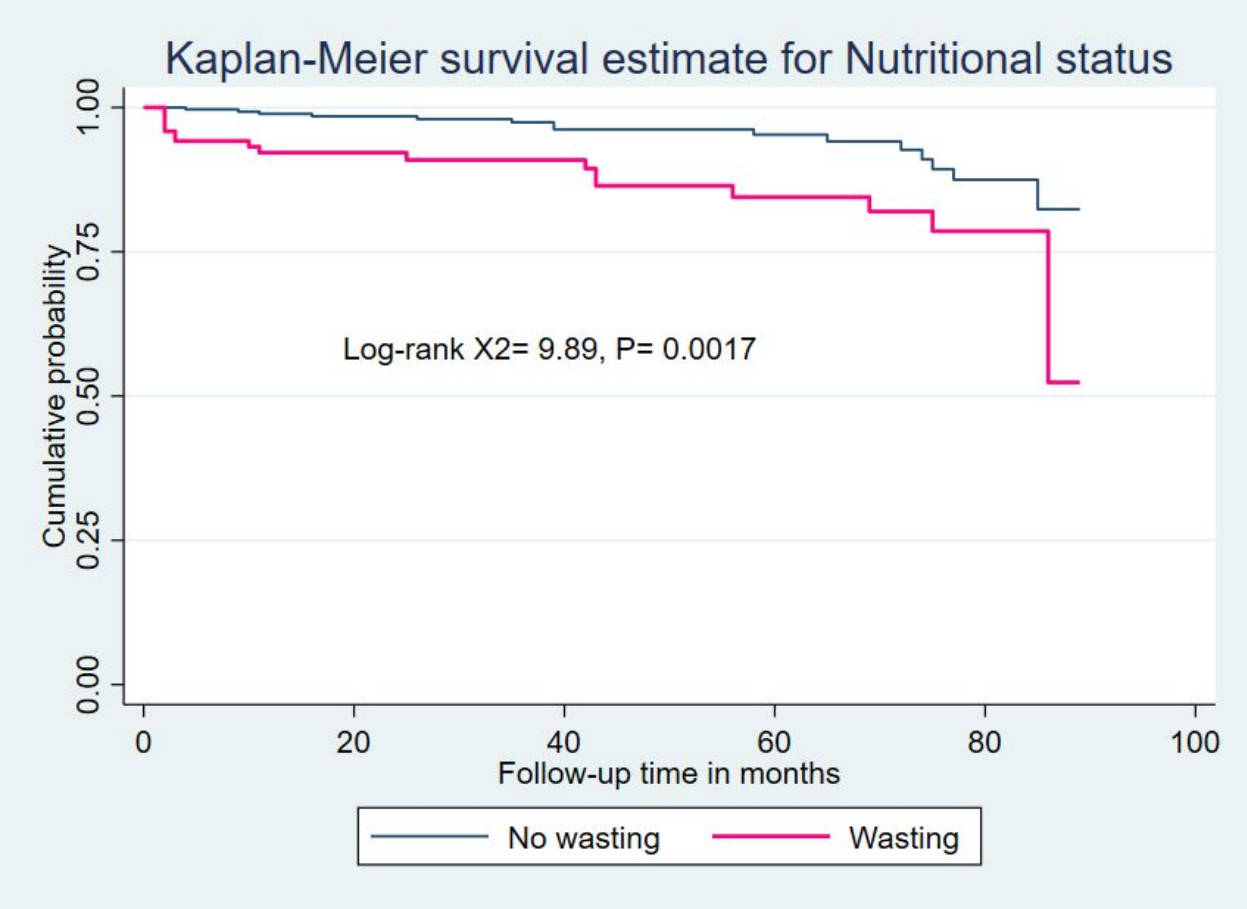
Supplementary Figure 2: Schematic diagram of the sampling procedure for among HIV-infected children on ART in Amhara Region Comprehensive Specialized Hospitals, Ethiopia



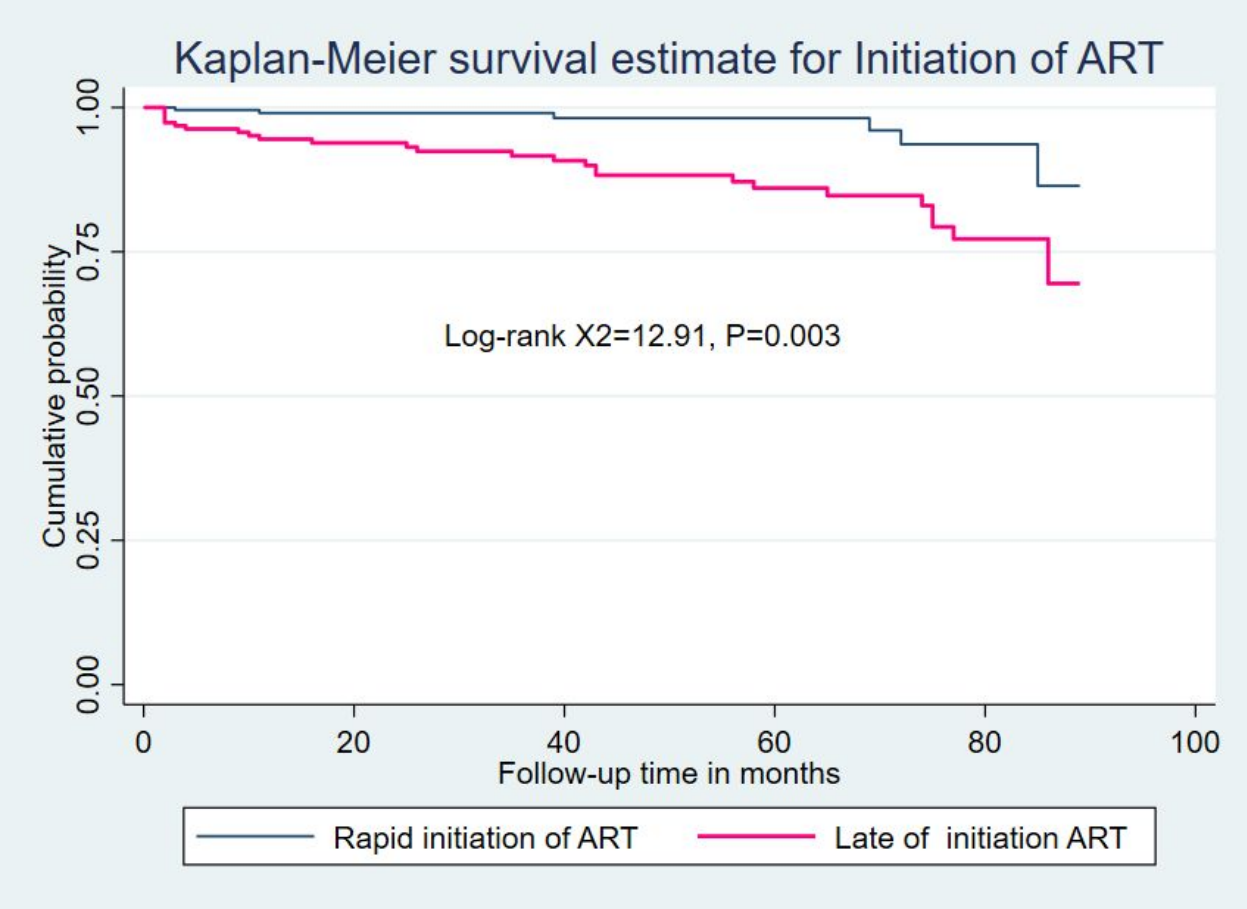
Supplementary Figure 3: Kaplan-Meier TB-free survival of anemic status a main predictor variables among children on ART in Amhara region comprehensive Specialized Hospitals, Ethiopia



Supplementary Figure 4: Kaplan-Meier TB-free survival of tuberculosis preventive therapy a main predictor variables among children on ART in Amhara region comprehensive Specialized Hospitals, Ethiopia



Supplementary Figure 5: Kaplan-Meier TB-free survival of Nutritional status a main predictor variables among children on ART in Amhara region comprehensive Specialized Hospitals, Ethiopia



Supplementary Figure 6: Kaplan-Meier TB-free survival of Initiation of Antiretroviral therapy a main predictor variables among children on ART in Amhara region comprehensive Specialized Hospitals, Ethiopia

Supplementary table 1: Baseline clinical, nutritional, and laboratory characteristics of HIV-infected children on ART in Amhara Regional State Comprehensive Specialized Hospitals, Ethiopia

Variables	Categories	Frequency	Percent (%)
CD4 cell count			
	Above threshold	**336**	**79.81**
	Below threshold	**85**	**20.19**
Anemia			
	No	**348**	**82.66**
	Yes	**73**	**17.34**
Functional status (n=299)			
	Working	**201**	**67.22**
	Ambulatory	**95**	**31.77**
	Bedridden	**3**	**1.00**
Developmental status (n=122)			
	Appropriate	**82**	**67.21**
	Delayed	**35**	**28.69**
	Regressed	**5**	**4.1**
Wasting			
	No	**299**	**71.02**
	Yes	**122**	**28.98**
Stunting			
	No	**218**	**51.78**
	Yes	**203**	**48.22**
Previous Opportunistic infections			
	No	**323**	**76.72**
	Yes	**98**	**23.28**
WHO Clinical staging			
	Stage I	**261**	**62.00**
	Stage II	**84**	**19.95**
	Stage III	**55**	**13.06**
	Stage IV	**21**	**4.99**
ART drug adherence level			
	Good	**296**	**70.31**
	Fair	**68**	**16.15**
	Poor	**57**	**13.54**
Tuberculosis preventive Treatment (TPT) taken			
	Yes	**267**	**63.42**
	No	**154**	**36.58**
Cotrimoxazole preventive Therapy (CPT) taken			
	Yes	**345**	**81.95**
	No	**76**	**18.05**
ART side effect			
	No	**275**	**65.32**
	Yes	**146**	**34.68**
Presence regimen change			

Yes	**214**	**50.83**
No	**207**	**49.17**
Initiated ART within seven days		
Yes	**231**	**54.87**
No	**190**	**45.13**
DTG contained ART drugs		
Yes	**69**	**16.39**
No	**352**	**83.61**

Supplementary Table 2: Summary of model comparison between Semi parametric and parametric Cox-regression model using log-likelihood, AIC, and BIC criteria

Methods	Cox-model	Weibul	Exponential	Gompertz	Lognormal	Log-logistic
Log-likelihood	-168.36	-139.6	-139.62	**-138.43**	-141.27	-139.96
AIC	294.64	240.79	238.94	**236.02**	238.19	240.53
BIC	355.28	309.52	303.62	**304.74**	306.92	309.26

* Akakie Information Criterion (AIC), Bayesian Information Criterion (BIC)