

Survival Analysis of Risk Factors for Mortality of Patients with Cardiovascular Diseases at Tikur Anbessa Specialized Tertiary Referral Hospital, Ethiopia: A Retrospective

Ashefet Agete¹, Girma Altaye², Ebrahim Talebi^{3*} 

Received: 19 Apr 2023

Published: 26 Feb 2024

Abstract

Background: Cardiovascular diseases (CVD) represent a leading cause of global mortality, necessitating proactive identification of risk factors for preventive strategies. This study aimed to uncover prognostic factors influencing cardiovascular patient survival.

Methods: This study, which used a sample size of 410, showed how to analyze data using simple random sampling. It was conducted at the Tikur Anbessa Specialist Hospital in Addis Ababa, Ethiopia, between September 2012 and April 2016. The Cox PH and stratified Cox regression models were used for the analysis.

Results: Findings disclosed a patient cohort where 200 patients (48.8%) persisted through subsequent evaluation, while 210 patients (51.2%) succumbed. Blood pressure (BP), specific CVD, and education levels (EL) exhibited nonproportionalities in scaled Schoenfeld residuals ($P < 0.001$), prompting necessary stratification. Inadequacies in the Cox proportional hazards model led to favoring the stratified Cox model. Notably, EL, BP, cholesterol level (CL), alcohol use (AU), smoking use (SU), and pulse rate (PR) exhibited statistical significance ($P < 0.001$). Acceptability of the absence of interaction in the model, with disease types as strata, was established. Different cardiovascular conditions served as distinct groups, where EL, AU, BP, PR, CL, and SU emerged as variables with statistically substantiated significance associated with the mortality of patients with CVD.

Conclusion: Implications stress the imperative of widespread awareness among policymakers and the public concerning cardiovascular disease incidence. Such awareness is pivotal in mitigating identified risk factors, guiding more effective healthcare interventions tailored to the multifaceted challenges posed by cardiovascular health.

Keywords: Cardiovascular, Cox proportional hazard model, Cox-Snell residual, Noncommunicable disease; Stratified Cox regression model

Conflicts of Interest: None declared

Funding: None

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Cite this article as: Agete A, Altaye G, Talebi E. Survival Analysis of Risk Factors for Mortality of Patients with Cardiovascular Diseases at Tikur Anbessa Specialized Tertiary Referral Hospital, Ethiopia: A Retrospective. *Med J Islam Repub Iran.* 2024 (26 Feb);38:20. <https://doi.org/10.47176/mjiri.38.20>

Introduction

Cardiovascular diseases (CVD) represent a collective of disorders affecting the heart and blood vessels, constituting a substantial global health concern responsible for 30% of all deaths (1). The World Health Organization's

2014 data underscored coronary heart disease as the leading global cause of mortality and a significant health burden (2). The escalating prevalence of cardiovascular disease in Africa, particularly in Ethiopia, has evolved into a

Corresponding author: Dr Ebrahim Talebi, Ebrahim.Talebi@iau.ac.ir

¹ Department of Statistics, Hawassa University, Ethiopia

² Department of Biology, Hawassa University, Ethiopia

³ Darab Branch, Islamic Azad University, Darab, Iran

↑What is “already known” in this topic:

Risk factors for cardiovascular diseases (CVD) comprise variables encompassing blood pressure, cholesterol levels, alcohol consumption, smoking habits, and pulse rate. Previous research has employed diverse survival models— including Cox proportional hazards, parametric proportional hazards, and accelerated failure time—to explore CVD mortality.

→What this article adds:

In this study, owing to a significant lacuna in the identification of factors associated with CVD patient mortality, the stratified Cox regression survival models were employed, yielding more suitable outcomes.

substantial public health issue with far-reaching socioeconomic implications (3). A significant portion of cardiovascular-related deaths occurs in low and middle-income countries, emphasizing the rising burden in these regions (4).

In Ethiopia, 34% of the population succumbs to non-communicable diseases, with a 15% prevalence of CVD, further compounded by cancer, chronic obstructive pulmonary disease, and diabetes mellitus (5). Urban populations in Ethiopia are experiencing increasing health concerns related to cardiovascular issues, including conditions such as obesity, elevated blood pressure (BP), dyslipidemia, heart ailments, and diabetes. Importantly, a research study conducted in Addis Ababa disclosed a prevalence of hypertension, a pivotal cardiovascular determinant, with rates of 31.5% observed in men and 28.9% in women (6). Amid the global epidemiologic transition, cardiovascular disorders are on the rise universally, overshadowed only by HIV/AIDS as the leading cause of death in sub-Saharan Africa, particularly among those >30 years old (7).

Motivated by the enduring prominence of cardiovascular disease as the primary global cause of mortality, particularly in developing nations like Ethiopia, this research addresses a significant gap in the current literature. While previous studies have employed various survival models, such as Cox proportional hazards (PH), parametric PH, and accelerated failure time models, to investigate CVD mortality, a critical void exists in identifying factors linked to patients' deaths from CVD using stratified Cox regression survival models (8). This research seeks to rectify this gap by employing a comprehensive analysis using stratified Cox regression models, aiming to uncover key determinants associated with cardiovascular patient mortality.

Methods

The research took place at Tikur Anbessa Specialized Tertiary Referral Hospital, situated on the medical campus of Addis Ababa University in Ethiopia. Secondary data constituted the basis of this investigation, encompassing a review of patient cards and information sheets. Data retrieval utilized the hospital's registration system, extracting information pertaining to CVD and patients' initial dates of follow-up entry. Given the nature of the data, it qualifies as survival data collected to investigate the time-to-event aspect. The observation involved a cohort of patients, with recorded times of either death or continuation of life. The event of interest could be mortality or instances of censoring. The study's target population consisted of cardiovascular patients investigated between September 2012 and April 2016.

The analysis was done using the stratified Cox regression model. To account for noncompliance with the proportional hazard assumption in specific covariates, the Cox regression model was also employed. This model incorporates covariates assumed to satisfy the PH assumption, while those requiring stratification are excluded. The stratified Cox regression model encompasses both interaction and noninteraction models, as detailed by Dunkler et

al (8). Ensuring methodological precision and a thorough understanding of the determinants impacting cardiovascular disease (CVD) patient mortality, this study underscores the critical utilization of stratified Cox regression models in data analysis. Moreover, comparative analyses will be conducted to elucidate and delineate prognostic markers intricately linked to CVD patient mortality, constituting the primary focus and contribution of this investigation.

Description of Data

This study utilizes secondary data obtained through a comprehensive review of patient records and information sheets. The utilized data were retrieved from the hospital registration system. The dataset under consideration qualifies as survival data, as it was systematically collected to investigate the time-to-event aspect. Typically, a cohort of patients is observed, and their respective times of death or lifetime are documented. The event of interest may be death, while instances where full information regarding the event is not available are considered censored. In the context of this study, cardiovascular patients who remained alive during the study period but were lost to follow-up before experiencing the event (death) are categorized as censored.

The study encountered several limitations, primarily stemming from the utilization of secondary data obtained from patients' medical records, which may be susceptible to incompleteness and bias. A notable constraint lies in the absence of comprehensive information on various factors known to potentially influence the survival time of cardiovascular patients. Variables such as physical inactivity, family history of CVD, dietary patterns, and socioeconomic factors, as indicated in the existing literature, were not available in the hospital records and, therefore, could not be incorporated into the study analysis. Another limitation arises from the single measurement of information at the commencement of follow-up, precluding the capture of potential changes or variations over time. Moreover, the lack of specific time intervals recorded during hospitalization constitutes a further limitation, hindering a more detailed analysis of the temporal dynamics within the study cohort. These limitations underscore the need for cautious interpretation of the study findings and highlight areas for consideration in future research endeavors.

Inclusion and Exclusion Criteria

This study focused on cardiovascular patients undergoing follow-up care throughout the designated study period, specifically targeting individuals aged ≥ 13 years. The inclusion criterion aligns with the hospital's standard practice of treating patients aged ≥ 13 . Consequently, patients <13 and those not actively receiving follow-up care were excluded from the study to maintain homogeneity within the selected cohort. This stringent inclusion criterion ensures that the study specifically addresses the characteristics and outcomes of cardiovascular patients within the specified age group under consistent follow-up care at the designated hospital, contributing to the internal validity and relevance of the study findings.

Selection of Variables

Explanatory variables, assumed to be risk factors for cardiovascular disease mortality, encompassed both categorical and continuous variables. While numerous factors could potentially influence the mortality of cardiovascular patients, this study selectively focused on specific variables due to limitations in data availability within patients' records. The examined variables included age at the commencement of treatment, sex, place of residence, EL, region, types of CVD, SU, AU, and BP at the start of treatment, PR at the start of treatment, and CL. It is essential to note that all these variables were measured and recorded at the initiation of treatment in the study area. The careful consideration of these variables aims to contribute to a nuanced understanding of the determinants associated with cardiovascular disease mortality within the specified cohort.

Survival Analysis

Descriptive survival analysis was conducted to assess and compare survival times across distinct groups within the study cohort. The log-rank test and Kaplan-Meier survival estimates were employed to investigate the statistical significance of variations in survival experiences among different groups defined by various factors. The null hypothesis subjected to testing posits no discernible difference in the probabilities of an event occurring at any given time point for each group of factor variables. This analytical method makes it easier to identify potential differences in survival outcomes linked to the given factor variables and allows for a thorough investigation of survival dynamics.

Model Checking in Cox Regression Model

Following the model fitting process, an evaluation of the fitted model's appropriateness is imperative, typically accomplished through the examination of model residuals. The Cox-Snell residual, introduced by Cox and Snell, serves as a metric for appraising the adequacy of the PH model fitness.

Stratified Cox Regression Model

The stratified Cox regression model represents an adaptation of the Cox regression model, facilitating control through the stratification of a covariate that deviates from satisfying the PH assumption. Covariates assumed to adhere to the proportional hazard's assumption are incorporated into the model, while those being stratified are not included as predictors. Within the stratified Cox regression model, both interaction and no-interaction models are delineated (8).

Statistical Software

The initial step involved a thorough examination of the data to ensure completeness and consistency. Subsequently, the data underwent a coding process before being entered into the computer system. To facilitate the descriptive analysis, frequency tables were generated using statistical software tools such as SPSS. For survival analysis,

the R programming language was employed, harnessing its capabilities to conduct in-depth survival analyses. In addition, STATA software was utilized for graphical analysis, enabling the creation and visualization of pertinent graphical representations to enhance the interpretability of the study findings. This systematic and software-intensive approach ensured the integrity of the data and facilitated a comprehensive exploration of the study variables through various analytical perspectives.

Results

Summary of Covariates

The investigation incorporated 410 patients from a cohort of 3300 patients aged >13 years, as identified from patient records. The log-rank test outcomes detailed in Table 1 indicate that sex, EL, SU, AU, specific cardiovascular disease (CVD) classifications, BP, and CLs are noteworthy covariates at a 5% significance level. Various levels of these covariates exert discernible influences on the survival duration of cardiovascular patients. Conversely, place of residence, region, and pulse rate (PR) do not exhibit a statistically significant impact at the 5% significance level. The findings presented in Table 1 also delineate the principal attributes of cardiovascular patients acquired from patient records within the study area. A total of 410 patients were included in the analytical framework. The functional status of patients is classified into 2 categories in this investigation: "alive" (censored) and "not alive" (event). Descriptive analysis reveals that among the 410 patients, 48.8% are alive throughout the study, while 51.2% are deceased.

Within the cohort of 410 cardiovascular patients under study, 232 patients (56.6%) were women, and 178 patients (43.4%) were men. Stratifying by sex revealed a death proportion of 191 patients (46.6%) in women, significantly lower than the 219 patients (57.4%) observed in men. Regarding ELs, 138 patients (33.7%) had no formal education, 101 patients (24.6%) completed primary education, 103 patients (25.1%) attained secondary education, and 68 patients (16.6%) held a college degree or higher. The highest death proportion was noted among patients with primary education (268 patients, 65.3%), followed by those with secondary education (203 patients, 49.5%), and the lowest proportions were observed for those with college or higher education (187 patients and 184 patients).

Analyzing types of CVD, the prevalence of coronary artery disease (IHD), CHD, RHD, stroke, HHD, and other CVD were 18.8%, 5.4%, 33.7%, 23.4%, 8%, and 10.7%, respectively. The highest mortality ratio was associated with coronary artery disease (IHD) at 71.4%, followed by RHD at 54.3%. Mortality rates for patients with CHD, other CVD, and stroke were 50%, 43.2%, and 41.7%, respectively, with the lowest rate of 30.3% observed in patients with HHD.

In terms of BP, 32.4% had normal BP, 61% had high BP, and 6.6% had uncontrollable BP. Patients with high BP exhibited the highest mortality rate at 61.6%, followed by patients with uncontrollable BP at 51.9%, and those with normal BP had the lowest mortality rate at 31.6%.

Table 1. Summary of covariates

Covariates	Categories	Event (%)	Censored (%)	Total	χ^2	DF	P-value
Sex	Female	108(46.6%)	124(53.4%)	232(56.6%)	5.5	1	0.019
	Male	102(57.3%)	76(42.7%)	178(43.4%)			
Place of residence	Rural	121(53.1%)	107(46.9%)	228(55.6%)	2.2	1	0.137
	Urban	89(48.9%)	93(51.1%)	182(44.4%)			
EL	Primary	62(44.9%)	76(55.1%)	138(33.7%)	14.4	3	0.002
	Secondary	66(65.3%)	35(34.7%)	101(24.6%)			
	Collage	51(49.5%)	52(50.5%)	103(25.1%)			
	Above	31(45.6%)	37(54.4%)	68(16.6%)			
Region	Addis Ababa	110(52.6%)	99(47.4%)	209(51.0%)	0.1	2	0.946
	Oromiya	54(49.1%)	56(50.9%)	110(26.8%)			
	Others	46(50.5%)	45(49.5%)	91(22.2%)			
SU	No	126(40.9%)	182(59.1%)	308(75.1%)	32.2	1	0
	Yes	84(82.4%)	18(17.6%)	102(24.9%)			
AU	No	98(39.7%)	149(60.3%)	247(60.2%)	27.5	1	0
	Yes	112(68.7%)	51(31.3%)	163(39.8%)			
Type of CVD	Coronary (IHD)	55(71.4%)	22(28.6%)	77(18.8%)	20.9	5	0.001
	Congenital Heart Disease	11(50.0%)	11(50.0%)	22(5.4%)			
	Rheumatic Heart Disease	75(54.3%)	63(45.7%)	138(33.7%)			
	Stroke	40(41.7%)	56(58.3%)	96(23.4%)			
	Hypertensive Heart Disease	10(30.3%)	23(69.7%)	33(8.0%)			
	Others	19(43.2%)	25(56.8%)	44(10.7%)			
BP	Normal	42(31.6%)	91(68.4%)	133(32.4%)	21.5	2	0
	High	154(61.6%)	96(38.4%)	250(61.0%)			
	Uncontrollable	14(51.9%)	13(48.1%)	27(6.6%)			
CL	Normal	83(36.9%)	142(63.1%)	225(54.9%)	27.5	1	0
	High	127(68.6%)	58(31.4%)	185(45.1%)			
PR	Regular	142(47.5%)	157(52.5%)	299(72.9%)	3.5	1	0.0062
	Irregular	68(61.3%)	43(38.7%)	111(27.1%)			

 χ^2 : Chi Square

For CLs, 54.9% had normal cholesterol, and 45.1% had high cholesterol. The mortality rate for patients with high cholesterol was 68.6%, whereas patients with normal CLs had a lower mortality rate of 36.9%. Regarding pulse count, 27.1% had an irregular pulse, and 72.9% had a regular pulse. Patients with an irregular pulse had a higher death rate at 61.3%, compared with those with a regular pulse, who had the lowest death rate at 47.5%. These comprehensive findings highlight the multifaceted impact of sex, EL, disease type, BP, CL, and PR on the mortality outcomes of cardiovascular patients within the study cohort.

Survival Analysis

Descriptive survival analysis was implemented to scrutinize and compare survival durations among distinct subgroups within the study cohort. Survival analysis incorporating both the log-rank test and Kaplan-Meier methodology estimates was employed to evaluate the statistical significance of variations in survival experiences among diverse factors. Figure 1 and Table 1 showcase the Kaplan-Meier estimator survival curve and log-rank test results, delineating the impact of categorical variables—including sex, EL, SU, AU, specific cardiovascular disease (CVD) classifications, BP, and CLs—on the survival time of cardiovascular patients. These analytical tools provide valuable insights into the disparate survival experiences associated with various factors, contributing to a comprehensive understanding of the dynamics influencing outcomes within the investigated population.

Cox Proportional Hazard Analysis

Initially, the Cox regression model was applied to the

dataset, and subsequent to investigating the proportional assumption, certain variables, namely types of cardiovascular disease, EL, BP, CL, AU, SU, and PR, were identified as statistically significant factors influencing the mortality of patients with CVD at a significance level of 0.05. Following this, the PH assumption was examined through a statistical test. This assessment involved determining the correlation between the Schoenfeld residuals for specific covariates and the log-log survival plot.

Table 2 delineates that variables including types of CVD, EL, and BP failed to satisfy the PH assumption. The P values of the rho statistic associated with these covariates were below 5%, signifying the rejection of the null hypothesis concerning the proportionality of the Cox proportional hazard model. In essence, the assumption of PH is not satisfied for these variables. Conversely, for CL, AU, smoking behavior, and PR, the P values exceeded 0.05. This suggests a lack of compelling evidence to reject the null hypothesis, signifying that these covariates satisfy the assumption of proportional hazard. This conclusion is further supported by the log-log survival plot, reinforcing the robustness of the PH assumption for CL, alcoholic use, SU, and PR.

Cox-Snell Residuals

Goodness of fit was assessed through the utilization of a Cox-Snell residual plot, as depicted in Figure 2. The plot depicts the Cox-Snell residuals estimation with the blue line, while the red line illustrates the origin with a slope equivalent to 1. The visual inspection of the plot raises concerns regarding the adequacy of the Cox proportional hazard (PH) model, as there is evident systematic deviation from the straight line. This observation is consistent

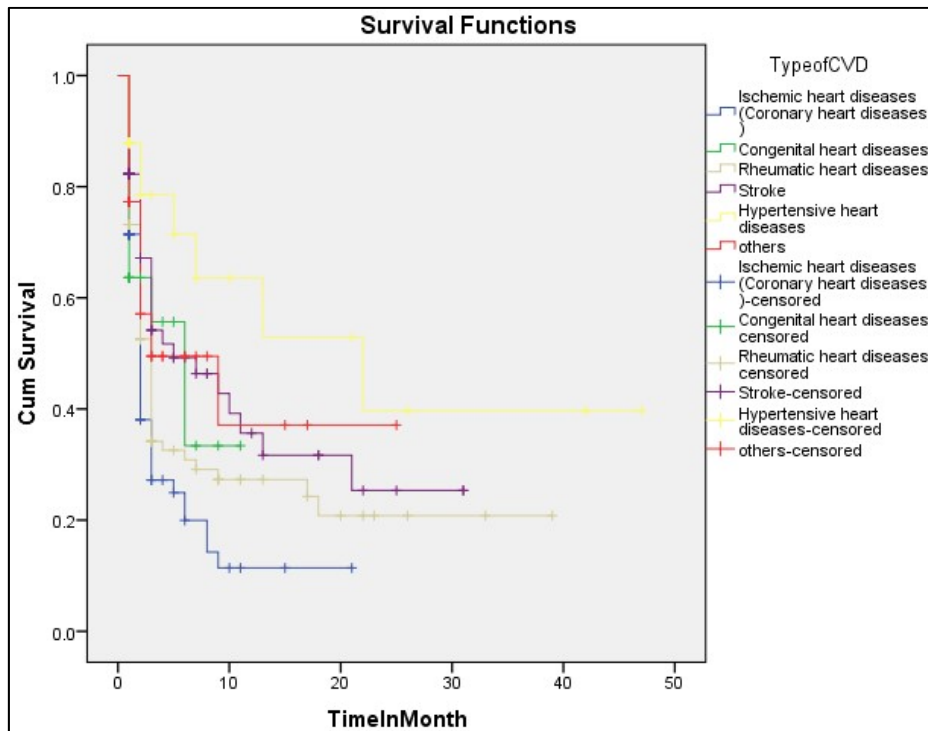


Figure 1. Kaplan-Meier survival estimate of categorical variables

with the findings from the scaled Schoenfeld residuals and log-log survival plot, both of which indicated non-proportionality for the variable recipient age. Given the cumulative evidence suggesting inadequacy of the Cox PH model, the stratified Cox regression model emerges as a more suitable alternative. The nonproportional nature of the data, as revealed by various diagnostic tools, underscores the necessity of employing the stratified Cox regression model for a more accurate analysis and interpretation of the survival data in the context of CVD.

Stratified Cox Regression Model

To address non-PH within the model, the Stratified Cox regression model was employed, presenting both noninteraction and interaction models. The forms of cardiovascular disease, BP, and EL were the strata that the noninteraction model classified; however, the interaction model allowed different coefficients on the included covariates for each of these strata. Evaluation of model adequacy involved comparing the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) for the

different models, as presented in Table 3.

Results indicated that the no-interaction model with types of CVD as strata was more suitable than the stratified Cox regression with other covariates as strata. Similarly, within the interaction model, the use of types of CVD as strata outperformed the alternative of using other covariates as strata.

Table 3 further provides values for AIC, BIC, and -2LL for both the stratified Cox regression with no-interaction and interaction models. Ultimately, based on AIC, BIC, and -2LL metrics, the stratified Cox regression model with no-interaction using types of CVD as strata emerged as the most appropriate for analyzing survival data in the presence of non-PH.

The final fitted stratified Cox regression model, with types of CVD as strata, is detailed in Table 4. Notably, the P values for EL, BP, CL, AU, SU, and PR were found to be statistically significant risk factors influencing the survival of cardiovascular patients. Specifically, Educ.Level1, BloodPress.1, Chole.Level1, AlcoholUse1, SmokUse1, and PulRate1 were identified as important levels contributing significantly to the mortality of cardiovascular patients at a 5% level of significance. Using both traditional and Bayesian survival analysis, this study at the Tikur Anbessa Specialized Tertiary Referral Hospital in Ethiopia sought to identify predictive markers influencing the survival of cardiovascular patients under treatment follow-up.

Discussion

The present investigation underscores the significant impact of EL on the survival of cardiovascular patients,

Table 2. Test Proportional hazard assumption by using scaled Schoenfeld residuals

Covariates	Rho	χ^2	P-value
Type of CVD	-0.00524	0.00665	0.035
EL	-0.09905	1.96551	0.051
CL	-0.00937	0.02019	0.887
AU	0.03608	0.34200	0.559
BP	0.09545	1.61336	0.024
SU	0.08095	1.65246	0.199
PR	-0.01128	0.02701	0.869

χ^2 : Chi Square

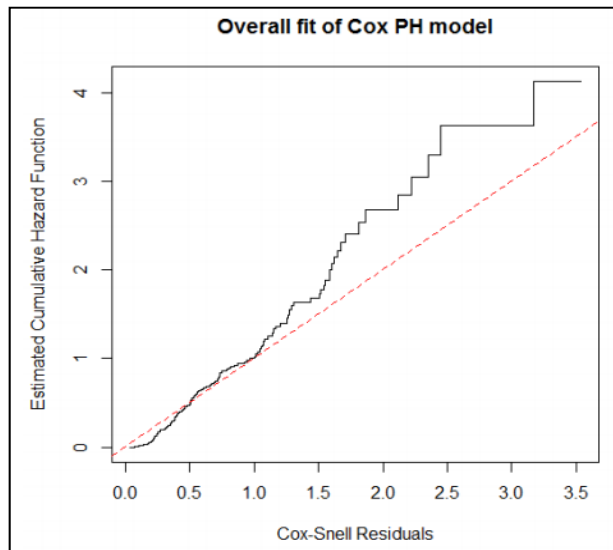


Figure 2. The Cox-Snell residual plot Cox PH model

Table 3. Result of comparison of stratified Cox regression with interaction and no-interaction models use the covariates education level, blood pressure and types of CVD as strata

Criterion	Stratified Cox regression models					
	No-interaction model			Interaction model		
	EL	BP	Types of CVD	EL	BP	Types of CVD
AIC	1580.161	1839.775	1500.89	1606.504	1847.063	1526.074
BIC	1624.338	1887.969	1537.035	1783.215	1991.645	1742.947
-2 LL	1558.161	1815.775	1482.89	1518.504	1775.063	1418.074

Table 4. Results of stratified Cox regression with no-interaction model, types of CVD as strata

Covariates	Categories	Coef	exp (cofe)	Se (cofe)	P-value	95% CI	
EL	1	0.3847	1.469	0.184	0.036	1.0248	2.1061
	2	0.1003	1.106	0.2	0.62	0.747	1.6362
	3	-0.4192	0.658	0.236	0.076	0.4139	1.0446
BP	1	0.6162	1.852	0.183	0.001	1.2942	2.6496
	2	0.0839	1.088	0.327	0.8	0.5732	2.0632
CL	1	0.5217	1.685	0.15	0	1.2566	2.2594
AU	1	0.4218	1.525	0.172	0.014	1.0894	2.134
SU	1	0.3731	1.452	0.167	0.026	1.0459	2.0164
PR	1	0.3763	1.4568	0.153	0.014	0.5085	1.9266

revealing that lower educational attainment is associated with a heightened mortality rate from CVD, as compared to those with a higher educational background (crude relative risk compared to high educational attainment) (9).

Furthermore, the study identifies smoking and AU as substantial risk factors contributing to a higher mortality risk among cardiovascular patients in contrast to their nonsmoker and nonalcoholic counterparts. These risk factors, although associated with other conditions, notably account for approximately 80% of deaths related to CVD (10). Notably, AU emerges as a predictive factor significantly influencing the survival of cardiovascular patients.

The research findings highlight the importance of BP control in cardiovascular patients, indicating that those with normal BP levels exhibit a lower risk of death compared to those with abnormal BP. This aligns with similar results from a study conducted in Ethiopia (11), which emphasizes the critical role of BP as an important cardiovascular disease risk factor for patient mortality (12).

PR, types of CVD, and smoking emerge as additional significant factors influencing the mortality of cardiovas-

cular patients. The study unveils that patients with irregular PRs face a 1.4568 times higher risk of death compared to those with regular PR, a finding consistent with prior research highlighting similar risk factors associated with increased mortality probabilities in cardiovascular patients (13).

Moreover, the study underscores the role of CLs in patient outcomes, revealing that those with high CLs are at a higher risk compared to those with normal CLs. This corroborates previous research establishing a firm link between elevated cholesterol concentration and increased mortality risk in patients with CVD (14, 15).

Conclusion

In the context of cardiovascular patients undergoing treatment follow-up at Tikur Anbessa Specialized Tertiary Referral Hospital in Ethiopia, this study aimed to ascertain prognostic factors influencing patient mortality. Stratified Cox regression models and Cox PH models were employed to analyze and model the survival time of cardiovascular patients. The survival times of cardiovascular

patients at Tikur Anbessa Specialized Hospital were found to be more suitably fitted by the stratified Cox regression models. Upon scrutinizing the stratified Cox regression model without an interaction model, using cardiovascular disease as strata, several variables emerged as statistically significant risk factors for cardiovascular patient mortality. These variables include smoking, alcohol consumption, BP, PR, CL, and EL. The findings of this suggest the need for heightened awareness among policymakers and the public regarding the current prevalence of CVD. This awareness is crucial for mitigating the identified risk factors associated with mortality, enabling healthcare professionals to implement targeted interventions to control these risk factors.

Abbreviations

CVD: Cardiovascular Diseases, NCD: Non-Communicable Diseases, PH: Proportional Hazards, IHD: Ischemic heart disease, CHD: Congenital Heart Disease, RHD: Rheumatic Heart Disease, HHD: Hypertensive Heart Disease, WHO: World Health Organization, HIV: Human Immune deficiency Virus, AIDS: Acquired Immune Deficiency Syndrome, AIC: Akakie Information Criterion, BIC: Bayesian Information Criterion, CI: Confidence Interval, LL: Log likelihood, PR: Place of residence, EL: Education level, SU: Smoking use, AU: Alcoholic use, TCVD: Types of CVD, BP: Blood pressure, CL: Cholesterol level, PR: Pulse rate.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Board of Tikur Anbessa Specialized Tertiary Referral Hospital, Addis Ababa, Ethiopia.

Authors' Contributions

Ashefet Agete designed the study and provided methodological expertise. Girma Altaye drafted the manuscript. Ashefet and Girma drafted the tables and figures and performed statistical analysis. Both authors read and approved the final manuscript. Ebrahim Talebi, with his guidance regarding the use of the desired statistical model, analysis of part of the data, review, and modification of the manuscript, submitted the manuscript by selecting a valuable journal.

Ethics Statement

This was not a study with human subjects; therefore, neither approval by the institutional review board nor obtaining of the informed consent was required.

Conflict of Interests

The authors declare that they have no competing interests.

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