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COLLEGE OF HEALTH SCIENCES
SCHOOL OF PUBLIC HEALTH
DEPARTMENT OF BIOSTATISTICS

JOINT MODELING OF ONSET OF MICROVASCULAR COMPLICATIONS AND LONGITUDINAL FASTING BLOOD SUGAR AMONG TYPE 2 DIABETES PATIENTS AT AYDER AND MEKELLE HOSPITALS, 2025

BY: TETEMKE MEKONEN, (BSc)

A THESIS SUBMITTED TO THE DEPARTMENT OF BIOSTATISTICS, COLLEGE OF HEALTH SCIENCES, MEKELLE UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE MASTER OF SCIENCE IN BIOSTATISTICS AND HEALTH INFORMATICS

JUNE, 2025
MEKELLE, ETHIOPIA

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JUNE, 2025
MEKELLE, ETHIOPIA

MEKELLE UNIVERSITY COLLEGE OF HEALTH SCIENCE
SCHOOL OF PUBLIC HEALTH DEPARTMENT OF BIOSTATISTICS

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Full title	Joint modeling of onset of microvascular complications and longitudinal fasting blood sugar among type 2 diabetes patients at Ayder and Mekelle hospitals, 2025
Duration of project	From; March 2024 to May 2025
Study Area	Ayder comprehensive specialized hospital and Mekelle General Hospital, Tigray, Ethiopia
Total cost of the project	25476.9 Ethiopian birr
Address of investigator	Email; tetie20007@gmail.com Cell phone; +251914890988

Advisor's Approval Sheet

This is to certify that the thesis entitled "Joint modeling of onset of microvascular complications and longitudinal fasting blood sugar among type 2 diabetes patients at Ayder and Mekelle hospitals, 2025" is submitted in partial fulfillment of the requirements for the degree of Master of Science (MSc) in "Biostatistics and Health informatics" to the Graduate Program of the College of Health Sciences of Mekelle University and has been carried out by: Tetemke Mekonen, ID No: CHS/PR169497/12 under my supervision. Therefore, I recommend that the student has fulfilled the requirements and hence can submit his thesis proposal to the Department of Biostatistics.

Kebede Embaye _____ _____

Name of Advisor Signature Date

Goitom Halefom _____ _____

Name of Co-advisor Signature Date

Declaration

I hereby declare that this MSc thesis is my original work and has not been presented for a degree at any other university, and all sources of material used for this thesis have been duly acknowledged.

Name: Tetemke Mekonen, Signature: _____ Date: __/__/2025

This MSc thesis proposal was submitted for examination with my approval as an advisor.

Name: _____

Signature: _____

Date: _____

Examiner Approval Sheet

We, the undersigned members of the Board of Examiners of the final open defense by "Tetemke Mekonen" have read and evaluated his/her thesis entitled "Joint modeling of onset of microvascular complications and longitudinal fasting blood sugar among type 2 diabetes patients at Ayder and Mekelle hospitals, 2025" and evaluated the candidate. This is therefore to certify that the thesis has been accepted in partial fulfillment of the requirements for the MSc. Degree in Biostatistics and Health informatics.

_____ _____ _____

Name of Chairperson Signature Date

_____ _____ _____

Name of Major Advisor Signature Date

_____ _____ _____

Name of Internal Examiner Signature Date

_____ _____ _____

Name of External Examiner Signature Date

Final approval and acceptance of the thesis are contingent upon the submission of the final copy of the thesis to the candidate's Department through the Office of the Department Graduate Program Coordinator.

Thesis Approved by

_____ _____ _____

Graduate Program Coordinator Signature Date

Certification of the final thesis

I hereby certify that all the corrections and recommendations suggested by the Board of Examiners are incorporated into the final thesis entitled “Joint modeling of onset of microvascular complications and longitudinal fasting blood sugar among type 2 diabetes patients at Ayder and Mekelle hospitals, 2025” by Tetemke Mekonen.

Kidanemariam Alem _____ _____

Department Head Signature Date

Stamp of the Department of Public Health

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ACRONYMS AND ABBREVIATIONS

ACSH	Ayder Comprehensive Specialized Hospital
AHR	Adjusted Hazard Ratio
AIC	Akaki Information Criteria
BIC	Bayesian Information Criterion
BMI	Body Mass Index
BP	Blood Pressure
CI	Confidence Interval
DN	Diabetic Nephropathy
DR	Diabetic Retinopathy
FBS	Fasting Blood Sugar
HDL	High Density Lipoprotein
HTN	Hypertension
IDF	International Diabetic Association
IQR	Interquartile Range
KM	Kaplan–Meier
LDL	Low Density Lipoprotein
LMM	Linear Mixed Model
MAR	Missing At Random
MICE	Multiple Imputation using Chain Equation
MGH	Mekelle General Hospital
PHA	Proportional Hazard Assumptions
SD	Standard Deviation
TG	Triglycerides
T2DM	Type 2 Diabetes Mellitus

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ABSTRACT

Introduction: Type 2 diabetes mellitus is a major cause of microvascular complications that impact patients' quality of life and contribute to long-term health issues. Worldwide, 18.8% of individuals with diabetes experience these complications, rising to 37.9% in Ethiopia. Fasting blood sugar levels are vital for managing Type 2 diabetes and are linked to the onset of microvascular complications; however, no studies have examined the relationship between fasting blood sugar progression and the onset of these complications in the Tigray region.

Objective: To jointly model the longitudinal pattern of fasting blood sugar and the time to onset of microvascular complications among type 2 diabetes mellitus patients at Ayder and Mekelle hospitals using a joint modeling approach, January 2018 - March 2024

Methods: A retrospective longitudinal study was conducted among 447 type 2 diabetes patients at Ayder and Mekelle hospitals from May 25 to July 10, 2024. Data were collected from patients' medical records using Kobo Toolbox, with training and supervision provided to data collectors. The adequacy of the random effects assumption was assessed and proportional hazards assumptions were validated. Joint modeling of Cox-PH with random intercept and random slope was fitted. Akaike Information Criteria and Bayesian information criterion were used for model comparison. Variables were interpreted via hazard ratios and 95% confidence intervals.

Result: The incidence rate of microvascular complications was 6 per 1000 person-months (95% CI: 5–7) with estimated restricted mean of 62 months. For one unit increased in longitudinal mean log (FBS), the hazard of complications was 18.92 (CI: 6.050, 54.598) times higher. For one unit increase in body mass index the hazard of complications was 6.1% more likely higher. A one-unit increase in the triglyceride level, the hazard of complications was 0.3% more likely higher. Patients on injection plus oral medications had a 2.45 times higher hazard of complications compared to those on single oral medications (AHR = 2.455, CI: 1.118, 5.302).

Conclusion: fasting blood sugar trajectory, baseline fasting blood sugar, Body mass index, Triglycerides level, proteinuria, and type of medication intake were significant predictors of time to onset of microvascular complications. These findings emphasize the importance of continuous monitoring and tight control of blood glucose levels in diabetes management to prolong the onset of microvascular complications.

Keywords: Type 2 diabetes mellitus, microvascular complications, fasting blood sugar, longitudinal study joint modeling

1. INTRODUCTION

1.1 Background

Type two diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by insulin resistance and relative insulin deficiency, which leads to chronic hyperglycemia. It leads to greater impairment in the metabolism of carbohydrates, lipids and fats and are observed not only in adults but also in younger individuals due to lifestyle factors. According to the World Health Organization and International Diabetic Federation (IDF), in the presence of symptoms such as polyuria, polydipsia and weight loss, the diagnosis of T2DM can be made on the basis of a random blood glucose concentration of ≥ 200 mg/dl. However, for asymptomatic patients, it is confirmed when the fasting plasma glucose concentration is ≥ 126 mg/dl 1,2]). The fasting blood sugar measurement is the concentration of blood glucose in milligrams per deciliter (mg/dl) after fasting for at least 8 hours (no caloric intake for 8 hours) [3].

T2DM is one of the most prevalent chronic diseases and the leading cause of both microvascular and macrovascular complications. According to the systematic review and meta-analysis, the pooled prevalence of microvascular complications among diabetic patients in Ethiopia was 32.9%. Among these, diabetic retinopathy affects 17.2%, neuropathy affects 10.5%, and nephropathy affects 11.5% of patients. Although there is no specific reports or pooled study of the study settings, the prevalence of microvascular complications in the Tigray region was 22.6% retinopathy, 19.17% nephropathy and 11% neuropathy [4,5].

Microvascular complications occur when small blood vessels in the body are damaged, whereas macrovascular complications occur when large blood vessels are damaged due to chronic hyperglycemia. The most common complications observed in T2DM patients are microvascular complications, which include retinopathy, nephropathy and/or neuropathy, all of which can lead to disability, dependency, accelerated patient morbidity and mortality [1,6].

Diabetic retinopathy occurs when tiny blood vessels found in the retina swell and leak or stop from the blood passing over, leading to vision impairment and blindness. It manifests as blurred vision and/or the presence of floaters [7]. Diabetic nephropathy (DN) occurs when structural and functional impairments (albuminuria or progressive loss of renal function) occur in the context of

diabetes mellitus [8]. Similarly, diabetic neuropathy is a common and serious complication of DM characterized by peripheral nerve damage due to chronic hyperglycemia, leading to symptoms such as pain, numbness, tingling, and muscle weakness [9].

Patients with T2DM who have uncontrolled fasting blood sugar (FBS) levels face a high risk of developing microvascular complications, mainly retinopathy, nephropathy and neuropathy. A report from the American Diabetes Association revealed an association between elevated FBS and microvascular complications [10]. Effective control of FBS for diabetic patients is mandatory for reducing the risk of diabetic complications. Interventions, including lifestyle changes and pharmacological treatments, are helpful in lowering FBS and preventing the progression of complications. Continuous monitoring of FBS levels is effective in improving patient outcomes [11].

1.2. Statement of the problem

T2DM is a significant public health problem globally and accounts for 90% of diabetes cases, with considerable impacts on human life and health expenditures. The most common concern among T2DM patients is microvascular complications (retinopathy, neuropathy, and nephropathy), which have a significant impact on patients' quality of life, morbidity, mortality and financial strains on individuals and the healthcare system. Globally, the magnitude of microvascular complications among T2DM patients has increased and varies significantly. A systematic review and meta-analysis across 38 countries revealed that the overall prevalence of microvascular complications in 2022 was 18.8% [12,13].

Microvascular complications are global public health concerns, and the incidence rates of these complications are substantial worldwide. Study among 4633 reported incidence rates of 92.8, 106.2, and 130.2 per 1000 person-years for retinopathy, neuropathy, and nephropathy, respectively, with increased rates associated with uncontrolled blood sugar and longer duration of diabetes. Additionally, in Ethiopia, the burden of microvascular complications among diabetic patients has increased, with a pooled prevalence of 32.89% [12,14].

Some factors associated with microvascular complications in T2DM patients include older age, long duration of diabetes mellitus, hypertension, obesity and residence, which have been documented in different studies [3,15,16]. Monitoring of longitudinal markers is essential to be aware of metabolic abnormalities and poorly controlled blood sugar, which increase the likelihood of microvascular complications [13].

However, given the high burden of microvascular complications in Ethiopia's Tigray region, no evidence links longitudinal FBS progression and time to onset of these complications. Most studies have focused mainly on cross-sectional or separate analyses and these types of studies ignored the interdependency between longitudinal process and time to event process. Specifically, they fail to estimate for how the change of FBS progression over time, influence for time to onset of microvascular complications [3,15,16]. Therefore, the study was aimed to fill these gaps by modeling the association between longitudinal FBS measurement and time to microvascular complications, to provide a more comprehensive understanding of how glycemic control impacts the progression of diabetic complications.

1.3. Rationale of the study

This study used joint modeling approach to analyze longitudinal FBS progression over time and time to onset of microvascular complications simultaneously among patients with T2DM at Ayder and Mekelle hospitals. Furthermore the study determined the prognostic factors for the longitudinal FBS progression over time and time to onset of microvascular complications. Hence, this research could be important in advancing new approaches for joint modeling and could inspire future research. In addition, it could be used as a potential reference for researchers conducting similar models.

The findings of this study could be used to improve patient management strategies by clarifying the impact of time-varying FBS levels on microvascular complications among T2DM patients. Furthermore, the findings of the study could be helpful for health workers to prioritize patients who are at risk of microvascular complications and tailor interventions to mitigate that risk to improve patient outcomes and medical expenses. In turn, the findings of the study may help program planners; policy makers, experts and other stakeholders set their plans and design strategies in a way that overcomes the prognostic factors for time to microvascular complications among T2DM patients.

2. LITERATURE REVIEW

2.1 Overview of Microvascular Complications

Different studies on microvascular complications related to T2DM patients have been conducted both globally and within Ethiopia. Numerous studies have indicated that microvascular complications are the most common complications among patients with T2DM, with diabetic neuropathy frequently identified as the most common complication. For example, a study conducted in Al-Madinah Al-Munawara among 275 T2DM patients reported that the incidence of microvascular complications was 31.27% [15], whereas a similar study in Dessie, Ethiopia, reported an incidence rate of 37.9% among 335 participants [16].

The variables associated with the time to occurrence of microvascular complications as well as with longitudinal measures of FBS among T2DM patients were age, sex, residence, systolic hypertension, diastolic hypertension, baseline FBS, type of medication and duration of the disease [13,14,17–20].

2.2 Literature on microvascular complications and longitudinal FBS

In California, a retrospective cohort study examined 135,199 newly diagnosed T2DM patients between 2003 and 2014. The median time to developing T2DM complications ranged from 3.0 to 5.2 years. Peripheral neuropathy had the highest incidence rate (26.9 per 1000 person-years), followed by chronic kidney disease (21.2 per 1000 person-years). Age, ethnicity and sex are significant factors associated with the time to occurrence of microvascular complications (21). This finding aligns with the findings of a study in Al-Madinah Al-Munawara, Saudi Arabia, involving 275 T2DM patients [15]. However, a study in Pakistan revealed that age and residence were not associated with the time to occurrence of microvascular complications [14].

In a retrospective follow up study conducted at the Felegehiwot and Debremarkos referral hospitals in northwestern Ethiopia between December 2014 and January 2020, 318 diabetic patients were enrolled and followed for 6 years. Survival and longitudinal sub-models were built. During the follow-up period, 26.3% of the patients with 95% confidence intervals (CIs) developed microvascular complications, and more than half of them experienced microvascular complications after 30 months of follow-up. According to the study age (Hazard Ratio(HR)=0.0027)) and urban residence (HR=-0.0597) were significant predictors of the time to

the occurrence of microvascular complications ([17]. A similar study at the university of Gondar involving 159 study participants revealed that these variables were significantly associated with the incidence of complications (22]. Similarly, a study from the University of Gondar referral Hospital among 341 newly diagnosed T2DM patients revealed that male sex (adjusted hazard ratio (AHR) = 0.50) was a significant predictor of the time to incidence of vascular complications [20].

Ten years duration of retrospective follow up study conducted in Wolaita and Dawuro Zone Hospitals, Ethiopia among 614 diabetic patients to assess predictors of time to diabetic nephropathy. According to a previous study, 93 patients developed diabetic nephropathy (DN) among 820048 person time, and the median follow up period of DN was 189.63 months. Multivariable Cox proportional hazards regression revealed that being illiterate (AHR = 2.21, 95% CI: 1.34–3.66) and living in urban dwellers (AHR = 2.25, 95% CI: 1.34–3.77) were significant predictors of the time to the occurrence of DN [19]. Another study conducted in governmental hospitals also revealed that age greater than 60 years was associated with the time to the occurrence of DN [23]. A longitudinal study implemented in Adama Hospital, Ethiopia, to evaluate the change in FBS over time and its predictors also revealed that secondary and higher education levels were significant (negatively) predictors of the FBS trajectory [24].

A retrospective follow-up study in Pakistan examined 4633 patients with T2DM over 11 years of follow-up, of whom 2336 (50.4%) were males. The incidence rates of microvascular complications in terms of neuropathy, retinopathy and nephropathy were 106.2, 92.8 and 130.2 per 1000 person-years, respectively. In this study, the incidence rate ratio (IRR) was estimated, which revealed that the incidence rate of microvascular complications was significantly greater among patients with a duration of diabetes >10 years, followed by those with a duration of diabetes 5–10 years and an HbA1c level >7% (IRR of retinopathy= 2.89; IRR of nephropathy =1.53) [14]. Studies in California and Dessie Hospital also revealed that a duration of DM > 5 years was a significant predictor of the incidence of microvascular complications among T2DM patients [16,21].

In Felege Hiwot referral Hospital, Ethiopia joint approach for longitudinal FBS and its determinants, as well as time to vascular complications, was projected among 159 T2DM

patients. According to the study, proteinuria (AHR = 1.62) and log FBS (1.453) were significantly positively associated with the time to occurrence of vascular complications. In additions to this duration of treatment, hypertension (HTN) and baseline FBS were significantly associated with the progression of FBS [22].

A retrospective cohort study was conducted at the University of Gondar referral hospital among 341 newly diagnosed T2DM patients with a median follow-up time of 81.5 months. Accordingly, the incidence of vascular complications was 40.6 cases/1000 person years of observation. In line with the findings of a study conducted at Felege Hiwot Hospital, proteinuria was significantly positively associated with the incidence of DM complications. Other significant clinical variables associated with the incidence of microvascular complications were high-density lipoprotein cholesterol (HDL-C) levels ≥ 40 mg per deciliter (mg/dl) (AHR = 0.43), low-density lipoprotein cholesterol (LDL-C) levels > 100 mg/dl (AHR = 3.05) and triglycerides > 150 mg/dl (AHR = 2.74) [20].

The joint approach for longitudinal FBS and time to occurrence of DR revealed that the type of medication, duration of DM since diagnosis and weight were significant predictors of changes in FBS levels, whereas the presence of hypertension, medication and duration were significant predictors of time to DR. This study revealed that a unit increase in the current value of FBS increased the hazard of developing DR by 1.35 [95% CI 1.12 1.63] times, whereas a unit increase in the rate of the FBS trajectory increased the hazard of DR by 1.70 [95% CI 1.21; 2.39] times. This finding confirmed that the longitudinal FBS trajectory was significantly associated with the incidence of microvascular complications among T2DM patients (25). Similar studies from governmental hospitals in the Harari Region and Northwest Ethiopia have shown that the baseline FBS and TG levels of diabetic patients were predicrors for time to microvascular complications [25,26].

According to a retrospective longitudinal study conducted in the Amhara regional state of comprehensive specialized hospitals, the baseline FBS in Northwest Ethiopia was significantly positively associated (AHR = 2.56 (95% CI: 1.68–3.92)) with time to diabetic neuropathy. Furthermore the study showed being anemic was significantly associated with time to diabetic neuropathy (AHR = 3.62, 95% CI: 2.46–5.33) [27].

A joint modeling approach was implemented in Northwest Ethiopia to assess the predictors of time to the occurrence of diabetic retinopathy (DR) and longitudinal FBS among 466 T2DM patients. Linear mixed effect models and Cox proportional hazard models were fitted separately and jointly. The participants were followed from February 2001 to 2016, and the incidence rate of DR was 2 per 100 person-years of observation, with a median follow-up time of 90.8 months. Current age ($\beta = -0.255$) and time were found to be significant predictors of changes in FBS [25].

A longitudinal study was implemented in Adama Hospital, Ethiopia, to evaluate the change in FBS over time and its predictors. There were 312 participants followed from September 1, 2018, to August 30, 2019, and a linear mixed model was applied. This reveals that the variable FBS was significantly above normal in all periods. On the basis of the linear mixed model, body mass index (BMI) and blood pressure (BP) were significantly (positively) associated with the FBS level, which was similar to the findings of studies conducted in California and Pakistan[8,9,15].

2.3. Conceptual Framework

This study aimed to investigate longitudinal FBS levels, the incidence of microvascular complications among patients with T2DM and its predictors and the associations between FBS levels and the time to event. According to studies conducted in different areas, these predictors can be grouped into socio-demographic, clinical and comorbidity factors.

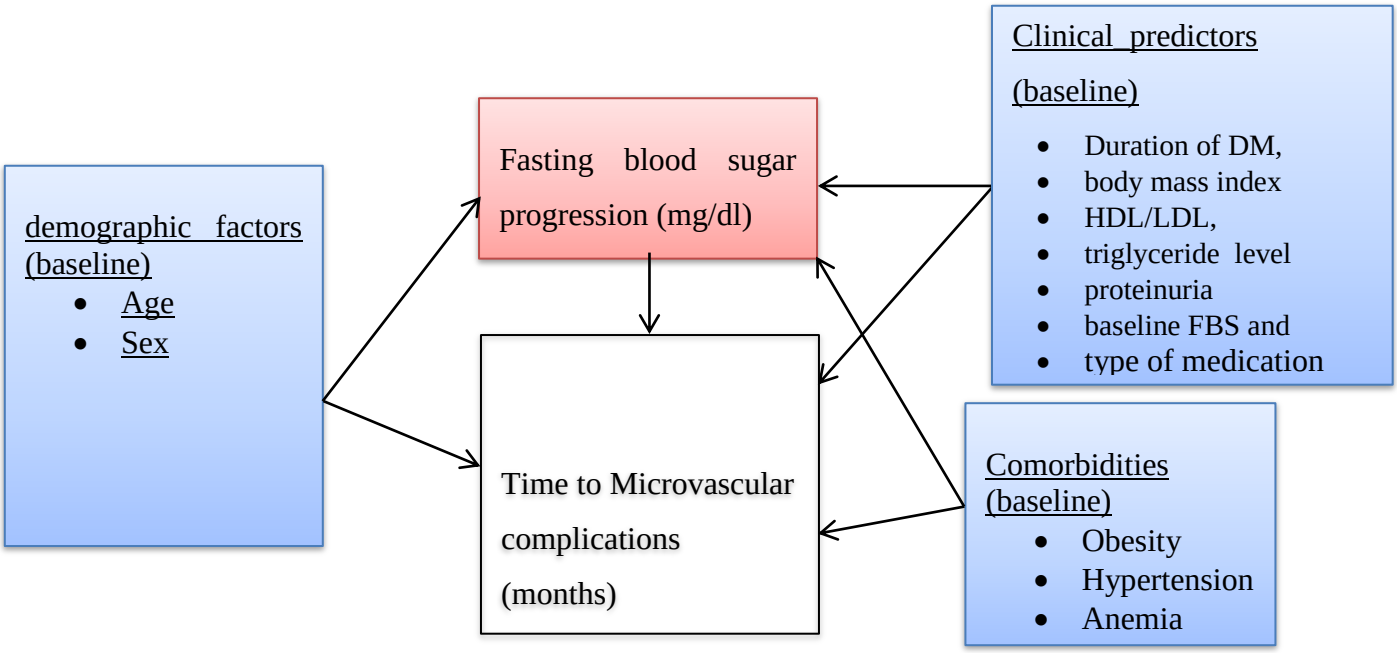

Figure 1: Conceptual framework for predictors and time to microvascular complications among T2DM patients in ACHS and MGH, Tigray Ethiopia, January 2018 –March 2024 (9-39).

3. OBJECTIVES

3.1. General Objective

To jointly model the longitudinal pattern of fasting blood sugar and the time to onset of microvascular complications among type 2 diabetes mellitus patients at Ayder and Mekelle hospitals using a joint modeling approach, January 2018 - March 2024

3.2. Specific Objectives

1. To determine time to onset of microvascular complications among patients with T2DM at ACSH and MGH, Mekelle, Ethiopia, January 2018-March 2024

2. To identify predictors that influences both FBS progression and risk of microvascular complications among patients withT2DM at ACSH and MGH, Mekelle, Ethiopia, January 2018-March 2024

3. To investigate the effect of FBS progression on time to microvascular complications among patients with T2DM at ACSH and MGH, Ethiopia, January 2018-March 2024.

4. MATERIALS AND METHODS

4.1. Study Area and Period

The study was conducted at Ayder Comprehensive Specialized Hospital (ACSH) and Mekelle General Hospital (MGH), both of which are governmental hospitals found in Mekelle, the capital city of Tigray region, northern Ethiopia. Mekelle is found 783 kilometers far away and north of Addis Ababa, the capital city of Ethiopia, with an elevation of 2084 above sea level. According to the United Nations Urbanization Prospects review, the population of Mekelle, 2024, was estimated at 611,574 [28].

Ayder Comprehensive specialized hospital is the most advanced tertiary care facility in the region. It serves as both a referral and non-referral hospital for patients from Tigray region and neighbour (Afar and Amhara) regions. It has four major departments and multiple specialty units, including a diabetic unit. It is equipped with advanced medical equipment's and diagnostic facilities. The hospital is staffed with diverse healthcare professionals, including clinical nurses, general practitioners, specialists, and subspecialists such as cardiologists, neurologists, urologists, and ophthalmologists.

Mekelle General Hospital (MGH) is also governmental in Mekelle city that provides comprehensive healthcare services, including chronic disease management, general medicine, surgery, obstetrics and gynecology. It is supported by a well-equipped laboratory and radiology department staffed by healthcare personnel starting from clinical nurses to general practitioners and senior physicians, including internists. Mekelle general hospital has separate clinics for diabetes mellitus.

The armed conflict in the Tigray region of Ethiopia from November 2020 to November 2022 has significantly damaged the health systems of the region**,** which has had a dire impact on the health and well-being of its inhabitants. According to the WHO Assessments on health facilities' functionality in 2024, 3.3% of all health facilities were fully damaged, whereas 86.1% were partially damaged. Due to significant disruptions in the transport and delivery of medications, patients faced a lack of medical supplies and laboratory functioning for their regular follow-up. [28]. Both hospitals also faced the attention of the health workforce resources needed for

diabetes management. The duration of follow up for this study was since January 2018 to March 2024 for a total of 6 years and data was collected from May 25 to July 10, 2024.

4.2. Study Design

A health facility-based retrospective follow-up study was conducted among T2DM patients.

4.3. Target and Study Populations

4.3.1 Target Population: All adult patients with T2DM attending medical follow-up at ACSH and MGH.

4.3.2 Study Populations: All adult patients with T2DM receiving medical follow-up at ACSH and Mekelle General Hospital (MGH) from January 2018 to March 2024 composed our study population. Both hospitals started to use the Diabetes Mellitus and Hypertension Cohort Register, which was prepared by the Federal Ministry of Health in January 2018. Accordingly, we decided to start the follow-up study in January 2018.

4.3.3 Study subjects: All adult patients with T2DM receiving medical follow-up at ACSH and Mekelle General Hospital (MGH) from January 2018 to March 2024 who had at least two follow up records of longitudinal FBS and with no microvascular complications at the start of their follow up.

4.4. Eligibility Criteria

4.4.1 Inclusion criteria: all adult patients who were newly diagnosed with T2DM and began follow-up at ACSH and MGH since January 2018.

4.4.2 Exclusion criteria: T2DM patients with only one baseline measurement of FBS or who developed microvascular complications before recruitment time to the study. Additionally, patients whose key clinical data, such as FBS data, were missing were excluded from the study.

4.5. Study Variables

4.5.1 Outcome Variables

The study has two outcome variables. For the longitudinal analysis, the outcome variable was FBS progression over time, measured in milligrams per deciliter. T2DM patients were monitored by measuring their FBS every three months during which their FBS values were recorded over a six-year follow-up period.

The second main outcome variable was time to onset of microvascular complications among T2DM patients measured in months. This variable was dichotomized in to event and censored. If the patient developed any of the three Microvascular complications (Diabetic retinopathy, nephropathy and neuropathy) during the follow-up period, it was recorded as event otherwise censored.

4.5.2 Predictor Variables

Socio-demographic variables: age and sex

Clinical variables: Duration of DM, body mass index (BMI), low-density lipoprotein (LDL), triglyceride (TG), proteinuria, baseline FBS level and type of medication
Comorbidities: hypertension, stroke, obesity and anemia

4.6. Sample Size Determination and Sampling

All adult patients with T2DM who initiated follow-up between January 2018 and December 2018 at ACSH and MGH, totally 447 individuals, were enrolled in the study. These patients were subsequently monitored until March 2024. The calculated minimum sample size required for the study was 408 T2DM patients. The log rank method of the sample size calculation formula is the most common method of sample size calculation for survival analysis. For unequal allocation, Schoenfeld's formula was used as follows.

The log rank method of the sample size calculation formula is given by [30].

$$n = \frac{2d}{2-\exp(-t\lambda_0)-exp^{(-t\lambda 1)}} \qquad 1$$

Where $d = \frac{(Z_{1-\alpha/2}+Z_{1-\beta})^2}{(p_x)\,(1-(p_x)\log(\Phi)^2}$ ---------- **2**

d- Number of DM patients with microvascular complications

Px = probability of participants having positive proteinuria

Φ = hazard ratio

λ_0 = hazard rate among participants with negative proteinuria

λ_1 = hazard rate among positive proteinuria

In a previous study conducted at Felege Hiwot and Debre Markos referral hospitals, North West Ethiopia, proteinuria variable had a larger sample size than the TG level. The predictor of proteinuria is a modifiable and proximal risk factor for T2DM-associated microvascular complications. Using power =0.8, α =0.05 and β=0.2, Φ= 1.518, Px of proteinuria positive = 0.34 & λ_1 (hazard rate among positive proteinuria) = 0.619, λ0 (hazard rate among participants with negative proteinuria) = 0.379 [17].

Table 1: Important predictors and parameters necessary to determine the sample size for T2DM-associated microvascular complications in ACSH and MGH, January 2018-March 2024 (n= 447)

Modifiable variable	Hazard ratio	$\lambda 1$	$\lambda 0$	P	1-p	D	Total sample size
proteinuria	1.518	0.619	0.379	0.34	0.66	204	408
TG level	2.11	0.72	0.45	0.13	0.87	125	252

$$d = \frac{(1.96+0.84\ )2}{0.34(0.66)[\log(1.518)]2} = 201$$

$$n = \frac{2*201}{2-\exp(-^{6*0.619})-exp^{(-6*0.379)}} = 204 \text{ for one group}$$

4.7. Data collection procedures

Diabetic patients receive medical follow-up every three months, and their measurements are recorded in their medical charts. All study participants who were newly diagnosed and started

their follow-up in January 2018 to December 2018 in both study settings were included in the study and followed for a period of 6 years. The data extraction tool was developed by reviewing the literature and guidelines related to factors associated with the DM and was deployed to the Kobo Toolbox. The secondary data were collected from patient medical records via a deployed data extraction tool using smartphones.

Data related to FBS levels were extracted from the patients' medical records at every follow-up during the course of diabetes. Information on the occurrence of microvascular complications was extracted from medical records, which involved identifying the date of diagnosis. Demographic, clinical and comorbidity-related variables were collected during the recruitment period (baseline). The collected data were expected to involve the study variables unless they were excluded from the study.

4.8. Data Quality Control and Management

The collected data were assessed for completeness and consistency by reviewing records and the content of patient medical records in the study area. The Kobo Toolbox was used to collect secondary data from medical charts of diabetic patients, which were helpful for controlling data entry errors. Data records that contained the necessary patient information were considered for the study. Three public health professionals were recruited as data collectors and trained for one day on the study variables, the subject matter, and data collection techniques using smartphones.

4.9. Operational definition

Time to microvascular complication: This refers to the duration from the initiation of follow-up for T2DM patients in either of hospitals until the diagnosis of any one of microvascular complications in months.

Microvascular complications: Type two diabetes mellitus patients with at least one of the following three DM-related complications (retinopathy, nephropathy or neuropathy) documented in their medical records during their follow-up period [3,16]. The data were dichotomized into having any microvascular complications as events and not having any microvascular complications as censored if the type two diabetic patients were free of any microvascular complications during the follow-up period.

Body Mass Index (BMI): BMI is a numerical value calculated from an individual's weight and height and is defined as the weight in kilograms divided by the square of the height in meters (kg/m^2).

Proteinuria: Presence of albumin protein in the urine typically measured by a urine dipstick test. It is defined as positive if the urine albumin concentration is > 30 mg/24 hours and negative if it is < 30 mg/24 hours [20,31].

Hypertension comorbidity was defined as having a history of taking antihypertensive drugs or having systolic blood pressure≥140 mmHg or DBP≥90 mmHg [20,32].

4.10. Data processing and analysis

Secondary data from medical charts were extracted using the developed Kobo Toolbox and exported, cleaned, edited, coded, and analyzed using Stata version 17 software. The data were prepared in both wide and long formats. The skewed distributions of longitudinal FBS measurements were transformed by log transformation to satisfy model assumptions, and multiple imputation methods involving multiple imputations by chained equations (MICE) were used to handle missing data. The data set was first changed to survival time format and then checked for the Cox proportional hazard assumption to determine the time-dependent covariate of the independent variable.

Missing data or unobserved data are common in longitudinal studies. Missing values can occur because of loss to follow-up, missed visits, data collection errors, malfunctioning laboratory equipment or participants missing specific time points. In joint modeling of longitudinal and time-to-event data, assumptions about the missing data mechanism are crucial for handling missing data appropriately. In the joint modeling approach, the assumption of missing at random (MAR) is handled without negatively affecting the validity of findings because it allows for the incorporation of observed values of covariates for estimating missing values [33]. Additionally, a joint model for longitudinal and survival sub-models, linked by shared random effects, was fitted using STJM package of Stata version 17.0 statistical software. Significant predictors were interpreted based on P-values and 95% confidence intervals (CI) at a significance level of 0.05.

4.10.1 Descriptive statistics

Descriptive measures such as relative frequency, percent, means, medians, standard deviations and interquartile ranges (IQR) were used to describe the data. Relative frequency and percentages were used to describe categorical predictors. The continuous predictors in this study were evaluated for their distributions before being summarized via measures of central tendency. The variables that followed a normal distribution were summarized using the mean and standard deviation. However, skewed continuous predictors were summarized using medians and IQRs.

4.10.1 Model Building

Rationale to Use Joint Modeling

In medical researches observations of subjects (for example, fasting blood sugar levels) are recorded at each follow-up time. Consequently, the onset of T2DM-associated microvascular complications was also recorded. We may not have complete records of longitudinal data for all subjects because some individuals develop T2DM-associated microvascular complications. Currently, the emerging field of research that fits these longitudinal and time-to-event models is called joint modeling. Separate analyses of longitudinal and survival data sometimes lead to biased inference because the time to event data may be associated with longitudinal trajectories. However, joint modeling analysis accounts for the association between them and provides valid and efficient inferences [34,35].

Joint modeling of longitudinal and time-to-event data is used to analyze repeated measurement data and the occurrence of an event of interest simultaneously. In addition to the improved efficiency and precision, it also handles missing data/informative censoring. Joint modeling provides robust estimates by incorporating all available information from partially observed longitudinal data and time-to-event data [35,36].

Hence, joint moding was used to identify predictors of changes in FBS levels and time to microvascular complications simultaneously. This approach consists of two interlinked sub-models: a longitudinal sub-model that captures the trajectory of FBS levels over time and a survival sub-model that models the time until the onset of microvascular complications. The joint modeling approach allows longitudinal data to enhance the understanding of survival outcomes

by accounting for the effects of changes in FBS levels on the risk of microvascular complications.

Linear mixed model

Linear mixed-effects models (LMMs) were used to analyze longitudinal FBS measurements collected from type 2 diabetes mellitus patients over multiple follow-up visits. Measurements of the same individuals are typically correlated; therefore, each individual in the population is expected to have his or her own subject-specific response pattern over time. The LMM model for longitudinal data accounts for between-person variability by estimating person-specific random effects around the parameters [37].

Repeated measures analysis of variance was used to test differences in the mean FBS across time points, with pairwise comparisons identifying specific intervals of significant change. The individual and mean profiles of longitudinal FBS levels were visualized graphically through line plots. The individual profile of the line plot was demonstrated by plotting each individual FBS level (Y-axis) against time (X-axis). Furthermore, the average profile of the line plot was shown by plotting the average FBS at each time point on the y-axis against time on the x-axis.

The LMM for the longitudinal FBS measurements captures the within-subject correlation due to repeated measurements taken over time. The notation and formula for the random intercept and slope LMM can be expressed as follows [37]:

At the population level

$$\boldsymbol{y_{ij} = \beta_0 + \beta_1 x_{1ij} + b_{1i} t_{1ij} + b_{0i} + \varepsilon_{ij}} \qquad 3$$

Where $\varepsilon_{ij} \sim N(0, \delta^2)$, $b_{0i} \sim N((0, \delta^2_{b0i})$

Sample level

$$\boldsymbol{\widehat{y_{ij}} = \widehat{\beta_0} + \widehat{\beta_1} x_{1ij} + \widehat{b_{1i}} t_{1ij} + \widehat{b_{0i}}} \qquad 4$$

- ✓ where $\boldsymbol{y_{ij}}$ is the FBS measurement value of patient i at observation time j.
- ✓ $\boldsymbol{\beta_1}$ indicates the vector of fixed effect coefficients of the population value
- ✓ $\boldsymbol{\widehat{\beta}}$ indicates the vector of fixed effect coefficients of the sample value

- ✓ $\boldsymbol{b_{0i}}$ indicates the vector of the random intercept effect
- ✓ $\boldsymbol{b_{1i}}$ indicates the vector of the random slope effect
- ✓ **X**- design matrix for fixed effects
- ✓ **t**- Indicated design matrix for random effects
- ✓ $\boldsymbol{\varepsilon_{ij}}$- the random error

The fixed effects (β_0 and β_1) in the random intercept and random slope model represent the population-level effects of the covariates on the FBS levels. The random intercept ($\boldsymbol{b_{0i}}$) tells us to what extent each subject's intercept deviates from the mean (group) intercept. The random slope ($\boldsymbol{b_{1i}}$) indicates how much the slope of a subject (i) deviates from the fixed slope ($\boldsymbol{\beta_1}$).

Checking the Adequacy of Random Effects Assumption

Variance of random effects: The variance for the random effect parameters of the fitted model was assessed to ensure adequate variability. The LMM output provided estimates of variances for random intercepts and random slopes. The substantial values for the random intercept reflected the variability in the baseline FBS levels across individuals; however, the variability for the random slope was small (0.37%) compared with the random intercept.

Model Diagnostics and Residuals: Important assumptions for the LMM were normality, linearity, homoscedasticity and independence of random effect parameters (Annex 11.3, p-56). We evaluated the distribution of the random effect estimates using residual plot to ensure that they followed a normal distribution with a mean of zero. The alignment of points along the reference line in the Q–Q plot indicated that the normality assumption was not violated. Furthermore, the independence of random effects from the residuals was examined by plotting the residuals against the estimated random effects. A lack of systematic patterns in the plot provided evidence that the random effects were independent of the residual variance.

Goodness of fit test: likelihood-ratio test was employed to assess the goodness of fit between the random intercept model and the random intercept with random slope model. The random intercept model was nested within the more complex model that included both random intercepts

and slopes. The likelihood-ratio test demonstrated that the random intercept with random slope model provided the best fit for the data.

Model Comparison: Model selection for the linear mixed models was guided by the Akaike information criterion (AIC) and Bayesian information criterion (BIC), which balance the model and complexity by penalizing the number of parameters. The AIC/BIC were used for nested and non-nested models. The optimal model identified was the LMM with both random intercepts and random slopes, as it yielded lower AIC/BIC values than did the model with only a random intercept.

Survival sub model

A survival sub-model was used to estimate the time to occurrence of microvascular complications among T2DM patients with possible predictors. A Kaplan–Meier survival curve was used to visualize patterns and compare survival time to microvascular complications between different groups of categorical predictors. The log rank test was used to identify significant categorical predictors for the time to occurrence of microvascular complications. The effect of each categorical predictor on the risk of occurrence of microvascular complications was checked by the log rank test**.** Variables with P values less than 0.25 from the log rank test and bivariable analysis were included in the final Cox proportional hazard model**.** the restricted mean was used to estimate the average time for which patients with T2DM were survive free of microvascular complications within the restricted period of the follow up period (72 months)

Cox-Proportional Hazard Assumption

The proportional hazard assumption assumes that the relative hazard of an event (occurrence of microvascular complications) remains constant across different time points. This means that the hazard ratio between any two individuals is the same at every time point (38). The PHA in the study was checked graphically and statistically.

Graphical techniques, which compare estimated -ln(-ln) survival curves across different categorical variables over time, were employed for all independent variables to determine

whether the relative hazard of microvascular complications remained constant over time. A parallel curve between the two comparison groups indicated that the PHA was satisfactory.

The PHA for all the predictors was also evaluated statistically. The Schoenfeld test was used to test statistically and examine whether the covariates had a time-varying effect on the hazard. While the graphical analysis indicated that certain predictors (marital status, hypertension, and obesity) were not parallel, the Schoenfeld residual test confirmed that the PHA was not violated, as their p values were greater than 0.5. Overall, the Schoenfeld residual test is regarded as a more robust and dependable method for assessing the PHA than the graphical approach. According to the Schoenfeld residual test, all the predictors met the assumptions except for obesity and proteinuria.

Using a time-dependent covariate, in this analysis of the Cox-PH assumption, both obesity and proteinuria violated the assumption because the P values from the Schoenfeld residual were less than 0.05, which were 0.034 and 0.028, respectively. To solve this failure of assumption, we add time interaction for proteinuria and obesity because they are endogenous covariates whose values can vary with time.

Furthermore, the assumption of no Multicollinearity was checked by examining the correlation matrix among the predictors and calculating the variance inflation factor for each predictor. Predictors with higher variable inflation factor (> 10) were removed to avoid redundancy among the predictors. The formula for the Cox PH model is as follows:
At the population level:

$$h(t,x) = h_0(t)e^{\sum_{i=1}^{p}\beta_i x_i} \qquad 5$$

$$\mathbf{h_1(t)}\Big/\mathbf{h_0(t)} = \boldsymbol{\Phi}$$

At the sample level:

$$\hat{h}(t,x) = \hat{h}0(t)e^{\sum_{i=1}^{p}\widehat{\beta_i}x_i} \qquad 6$$

- ✓ $h0(t)$ - the baseline hazard function
- ✓ $ho(t)$- the hazard of group zero, $h1(t)$-the hazard of group one
- ✓ β-regression coefficient
- ✓ p - number of parameters

- ✓ Φ is the hazard ratio

Joint modeling

The final joint model formally associates longitudinal FBS with the time to occurrence of microvascular complications. This framework models the hazard of experiencing an event as dependent on a subject-specific characteristic of its longitudinal trajectory. The final joint model was developed by integrating the LMM with a random intercept and random slope for longitudinal FBS and the Cox PH model for the time to microvascular complications. More specifically, we have:

$$\widehat{\boldsymbol{y}_{\iota J}} = \widehat{\boldsymbol{\beta}_{\mathbf{0}}} + \widehat{\boldsymbol{\beta}_{\mathbf{1}}}\boldsymbol{x}_{\mathbf{1}\boldsymbol{ij}} + \widehat{\boldsymbol{b}_{\mathbf{1}\boldsymbol{\iota}}}\boldsymbol{t}_{\mathbf{1}\boldsymbol{ij}} + \widehat{\boldsymbol{b}_{\mathbf{0}\boldsymbol{\iota}}}$$

$$\widehat{h_{\iota}}(t) = \widehat{h_0}(t)exp(^{\sum_{i=1}^{p}\widehat{\beta_{\iota}}x_i} + \hat{\alpha}\widehat{y_{ij}}) \qquad 7$$

- ✓ $\widehat{h_{\iota}}(t)$ represents the estimated hazard function for the i[th] individual at time t
- ✓ α *is the* strength of the association between longitudinal FBS and the risk of microvascular complications

Alpha (α) is an association parameter used to assess the association between longitudinal FBS changes and time to microvascular complications. This value indicates the value of the FBS level at which microvascular complications occurred, and for any change in the mean FBS level, the timing of microvascular complications was determined. This value was interpreted as the hazard of microvascular complications increased by an exponent of alpha (exp^{α}) times per unit increase in the FBS measurement. The association structure used in standard joint modeling is time-dependent association. The random intercepts and slopes were shared between the two models and were dependent on the longitudinal value of FBS and the onset of T2DM-associated microvascular complications.

Before separate linear mixed and survival models were developed, bivariable analysis was conducted to identify potential candidate predictors. Multivariable analysis helps to control potential confounders and analyze the effect of a factor in the presence of other factors. In the

For multivariable joint modeling, a covariate with a p value of < 0.05 that contained in the 95% confidence interval was considered significant covariates. In the final Cox proportional hazard model, the hazard ratio was interpreted as follows: for any change or unit increase in the predictors, the hazard of microvascular complications was increased or decreased by the amount of hazard ratio from the base line. Longitudinal and survival sub models were fitted jointly.

4.11 Ethical Considerations

Ethical approval for this study was obtained from the Institutional Ethical Review Board of Mekelle University College of Health Sciences, along with the necessary approval and supporting letter. Since the study utilized secondary data, there was no direct patient involvement. To ensure confidentiality, all patient information was anonymized before data collection. Data were entered into the Kobo Toolbox, which was protected by password encryption to prevent unauthorized access. No identifying information was disclosed or shared. Furthermore, the use of secondary data posed no potential harm to the study subjects or the hospital, as the data were used solely for research purposes.

4.12 Plan for Dissemination of Study Finding

Upon completion of this research project, the results will be submitted and presented to the School of Public Health, College of Health Sciences, Mekelle University. After approval, the findings will be disseminated to key stakeholders, including Ayder Comprehensive Specialized Hospital, Mekelle General Hospital, the Tigray Regional Health Bureau, and other relevant organizations. Dissemination will occur via both email and hardcopy to ensure that the study's findings are formally shared and can be utilized by health planners for future interventions and research. Finally, the research findings will be disseminated to journals for publication to make the study accessible to a broader audience.

5. RESULTS

Descriptive statistics

A total of 454 patients medical record from patients with T2DM who attend their follow-up at Ayder Comprehensive Specialized Hospital (ACSH) and Mekelle General Hospital (MGH) were reviewed, of which 7 (1.6%) were excluded because of incomplete medical records. Finally, 447 (98.5%) medical records were left in the final analysis. Some of our independent variables had missing values: LDL was missing for 6 (1.2%), HDL was missing for 5 (1.1%) and TG levels were missing for 3 (0.6%). Females accounted for nearly half of the total participants. A significant proportion of these patients developed microvascular complications during follow-up. Descriptive statistics for the study participants are summarized below

Table 2: Descriptive statistics for categorical predictors included in the study of T2DM patients at ACSH and MGH, January 2018 to March 2024 (n=447).

Variables	Category	Outcome status		Total (%)
		Censored (%)	Microvascular Complications (%)	
sex	Female	150 (67.6)	72 (32.4)	222 (49.7)
	Male	157 (69.8)	68 (30.2)	225 (50.3)
Proteinuria	Positive	48 (38.4)	77 (61.6)	125 (28)
	Negative	259 (80.4)	63 (19.6)	322 (72)
history of hypertension	Yes	91 (59.9)	61 (40.1)	152 (34)
	No	216 (74.2)	79 (26.8)	295 (66)
Type of medication	Injection plus oral agent	52 (47.7)	57 (52.3)	109 (24.4)
	Injection alone	9(64.3)	5(35.7)	14 (3.2)
	More than one oral agent	128 (65)	69 (35)	197 (44)
	One oral agent	118 (92.9)	9 (7.1)	127 (28.4)
Obesity	Yes	33 (55.9)	26 (44.1)	59 (13.2)
	No	274(70.6)	114(29.4)	388 (86.8)
Anemia	Yes	18 (36)	32 (64)	50 (11.2)
	No	289 (72.8)	108 (27.2)	397 (88.8)

The median age of the participants was 60 years (interquartile range (IQR) = 57, 67), with a median weight of 66 kg (IQR = 60, 72). The median body mass index (BMI) was approximately 26 kg/m^2 (IQR: 21.9, 30.1). Blood pressure readings revealed a median diastolic pressure of 80 mmHg (IQR = 75, 90) and a median systolic pressure of 127 mmHg (IQR = 115, 146). The mean hemoglobin level was approximately 14 g/dl (SD =

2.0 mg/dl), whereas the baseline fasting blood sugar level was typically approximately 178 mg/dl (IQR = 152, 231), with a wide range observed.

Table 3: Descriptive statistics for continuous predictors included in the study of T2DM patients at ACSH and MGH, January 2018 to March 2024 (n= 447)

variables	mean	median	Standard Deviation	IQR
age		60		15
weight		66		12
BMI		25.7		4.1
BP diastolic		80		15
BP systolic		127		31
Baseline Hgb (n= 446)	17.2		2.0	
Baseline FBS		176		76
TG level ((n= 444)		140		69
HDL(n= 442)		37.6		19
LDL (n= 441)		114		73

Further analysis of metabolic markers revealed a median triglyceride (TG) level of approximately 140 mg/dl (IQR = 113,182) and a median high-density lipoprotein (HDL) value of 37.56 mg/dl (IQR = 31, 50). Furthermore, the median low-density lipoprotein (LDL) level was 114 mg/dl (IQR = 88, 161 mg/dl) (see above).

Longitudinal Sub Model (Linear Mixed model)

Summary of Longitudinal FBS

The median FBS at baseline (time 0) was 176 mg/dl, with an IQR of 79 mg/dl, and decreased to at second visit (month 3) 157 mg/dl, with an IQR of 75 mg/dl. The median FBS progressively decreased over time to 123 mg/dl, with an IQR of 37 mg/dl (for more information, see

Table 11).

The longitudinally measured FBS level was tested for normality assumptions using a histogram, and the measured value was right-skewed. As displayed in

Figure 2, the data were skewed and not normally distributed. Hence, it was transformed via log transformation because it had a small chi-square value. After being transformed, the distribution became normal.

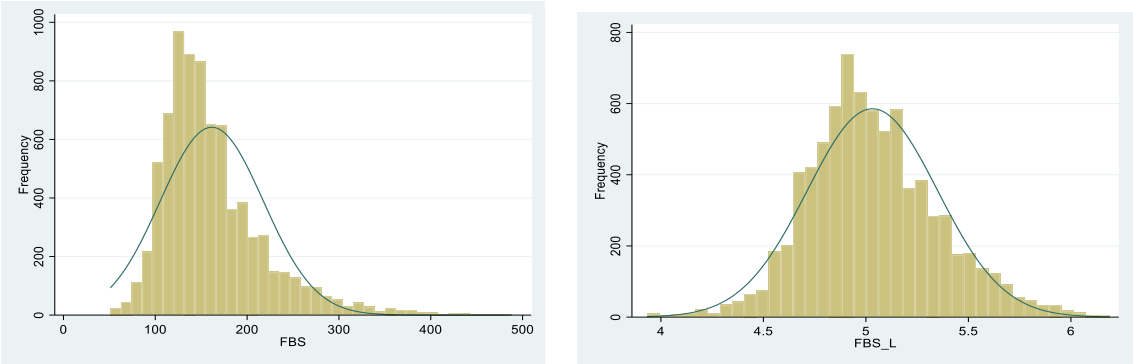

Figure 2: Histogram for normality assumptions of FBS before (left) and after log transformation (right) for patients with T2DM at ACSH and MGH, January 2018 to March 2024 (n=447).

Exploratory analysis was used to visualize the patterns of individual profile plots and the average evolution of FBS over time. The plot **(**Figure 3**)** shows wide individual variability in the FBS measurements at baseline and was almost parallel over time. The connected lines for each individual show variability in FBS levels across the time points. Some subjects exhibit fluctuating patterns, whereas others maintain relatively stable measurements. The mean FBS line rapidly decreased in the first 6 months and varied over time.

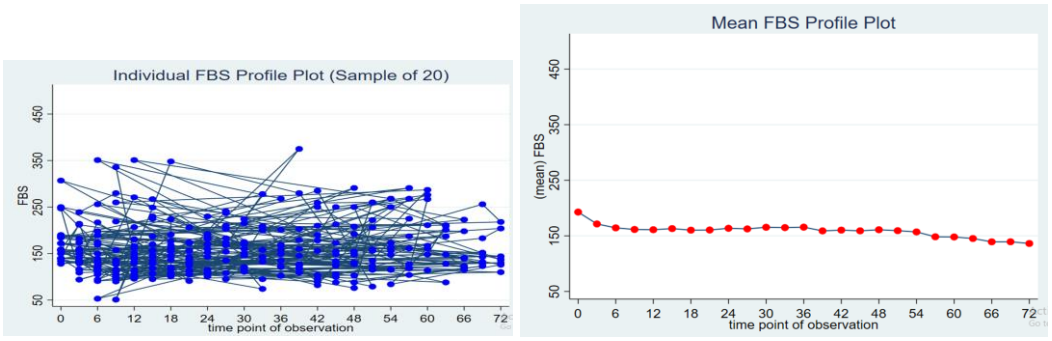

Figure 3: Individual (left) and mean (right) profile plots of FBS over time for patients with type 2 diabetes at ACSH and MGH from January 2018 to March 2024 (n = 447)

Repeated measures analysis of variance demonstrated that there was a significant difference in the mean FBS among the different time points of the observations, as the F value was significant ($p < 0.001$).

Bivariable linear mixed models: Variables with a p value less than or equal to 0.25 in the bivariable analysis were considered for inclusion in the multivariable. accordingly, the predictors: age, sex, weight, baseline hemoglobin, FBS at baseline, triglycerides, HDL, LDL, type of medication, anemia, and proteinuria were associated with longitudinal FBS levels.

Model comparison: the LMM with random intercept and random slope was best fit than the models with only fixed parameters (marginal model) and LMM with random intercept only models because the AIC/BIC value for LMM with both random intercept and random slope was smaller than others

Table 4: Summarized value of AIC/BIC for T2DM Patients data at ACSH and MGH, January 2018-Marchr 2024 (n=447)

Title	Models	AIC	BIC
Models for longitudinal Analysis	Fixed model (LR)	3507.28	3583.949
	Random intercept LMM	1724.604	1822.183
	Random intercept and slope LMM	1471.942	1576.491

Multivariable Linear Mixed Model

The LMM results revealed that the standard deviation for the random slope was 0.0037, indicating that the variability in the mean FBS progression over time among individuals with T2DM, varies little or was almost parallel. The random intercept was 0.181, which indicated that 18.1% of the variation in FBS among T2DM patients was at baseline. The residual error of 0.254 indicated 25% variation between the observed FBS values and the predicted FBS values among T2DM patients. The observation time was significantly associated with the FBS level; over time, the mean log(FBS) was decreased over time by 0.2%.

The multivariable analysis of the LMM demonstrated time, sex, TG levels, medication types, anemia, HTN and proteinuria were significant determinants of FBS progression. For a unit increase in TG level the mean log (FBS) of patients with T2DM was significantly increased by 0.004 ($p = 0.009$). Similarly females were expected to have an approximately 3.98% higher than those males holding other variables constant.

The mean FBS trajectory among Patients on injection alone was increased by 17.12% compared to those who were on single oral agent. Similarly the mean FBS among patients taking injection

and oral agent simultaneously was raised by 19.72% (p <0.001). Anemia was linked to a coefficient of 0.095, suggesting that anemic patients among T2DM were with raised FBS levels by 9.97% (p < 0.01). Patients with proteinuria were expected to have approximately 3.3% higher FBS levels than patients with negative proteinuria holding other variables constant.

Table 5: Predictors of the FBS trajectory among T2DM patients at ACSH and MGH from January 2018 to March 2024 (n = 447)

Variables	estimates	Standard error	[95% CI]		P- values
			Lowe CI	Upper CI	
Time point	-0.002	0.0003	-0.0022	-0.0012	0.001
Sex male (Ref)					
Sex female	0.031	0.0193	-0.006	0.072	0.115
TG level	0.004	0.0001	0.0009	0.0062	0.009
HTN history (Ref = No)					
HTN history Yes	-0.044	0.0218	-0.0867	-0.0011	0.044
Medication (Ref=only one)					
Medication (Injection alone)	0.110	0.0572	-0.0015	0.2223	0.053
Medication (Injection plus oral)	0.165	0.0277	0.1104	0.2188	0.001
Medication (More than one)	0.100	0.0236	0.0545	0.1470	0.001
Obesity (Ref = No)					
Obesity Yes	0.009	0.0295	-0.0484	0.0671	0.751
Anemia (Ref = No)					
anemia Yes	0.075	0.0316	0.0127	0.1366	0.018
Proteinuria negative(Ref)					
Proteinuria positive	0.055	0.0228	0.0101	0.2000	0.016
Constant	4.920	0.0283	4.8650	4.9761	0.001
Random effects	**Estimates**		**95% (confidence Interval)**		
SD(slope)	0.0037		0.0002	0.0042	
SD (Intercept)	0.1810		0.1667	0.1967	
SD (Residual) error	0.2426		0.238	0.2467	

Formula for calculating the change in the FBS for the longitudinal process = ($e^{estimates}$-1), Ref=reference

Survival sub-model

Time to microvascular complications and its incidence

Patients with T2DM were followed for different periods of time: a minimum of 6 months and a maximum of 72 months, with a median follow-up time of 72 months and an IQR of 26 months (46, 72). They were followed for a cumulative duration of 23556 person–months of observation.

The incidence rate of microvascular complications was 6 (95% CI, 5–7) per 1000 person–months of observation or 72 per 1000 person-years of observation.

KM Estimates: This study revealed that the median time to microvascular complications was not within the observed follow-up time range because the data were right censored (

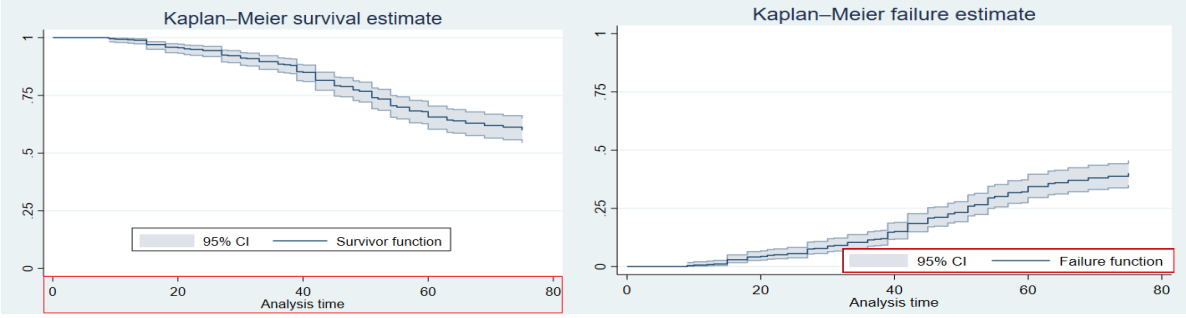

Figure 4). The restricted mean was 62.8 (95% CI; 60.94–64.58) months indicated that, on average, patients with T2DM survived free of microvascular complications for 62.8 months within the 72-month follow-up period.

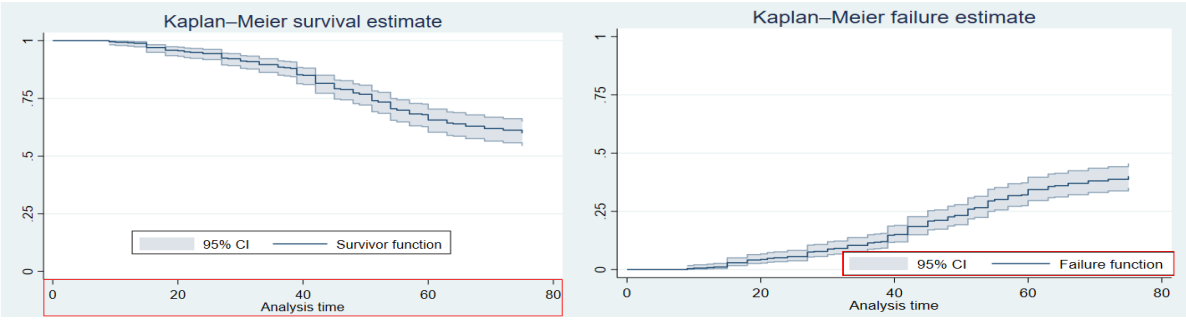

Figure 4: Median survival time for right-censored data for patients with T2DM at ACSH and MGH from January 2018 to March 2024 (n = 447)

The KM curve for the proteinuria test among T2DM patients showed that the survival probability of patients with proteinuria was lower than that of patients who were negative for proteinuria. The log rank test confirmed that there was a statistically significant difference between the two groups (P (chi^2) <0.001). Similarly, the log rank test for anemia reported that there were

statistically significant differences between the two groups, as the p value in the log rank was 0.001. Furthermore, the KM survival estimates for time to any of microvascular complications among T2DM patients on the basis of sex was displayed, and there were no statistically significant differences between each group of predictors (P (chi^2) =0.7256 and =0.14, respectively) (for more see **Table 8**).

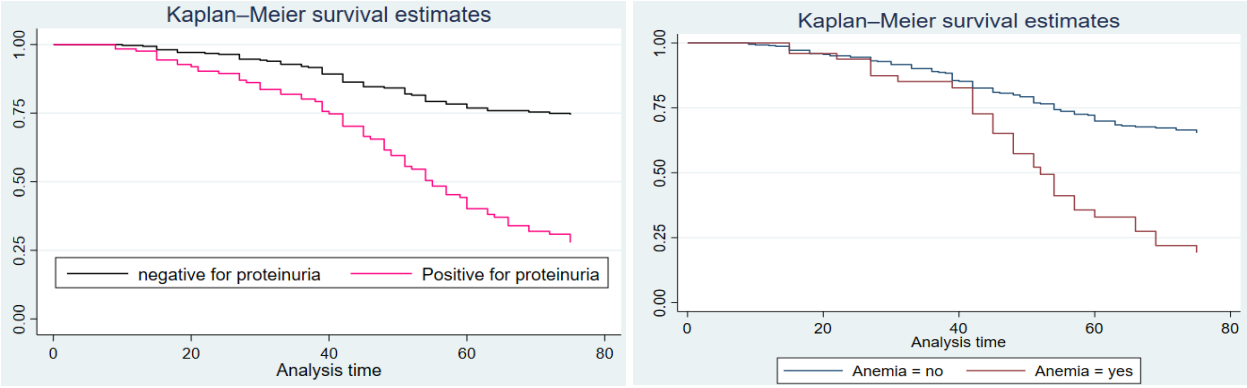

Figure 5: KM survival estimates for the two groups of patients with proteinuria (left) and Anemia (right) among T2DM patients at ACSH and MGH from January 2018 to March 2024

Cox-Proportional Hazard Assumption (Cox-PHA): The graphical visualization of predictors such as sex and residence seems that lines were crossed; however, statistically, with the Scheinfeld Residual, they fulfilled the Cox-PHA (p values were 0.34 and 0.79), respectively. The graphical presentation below shows that the graphs for proteinuria seem parallel; however, the P value derived from the Scheinfeld residual test was 0.026, which indicated that the PHA was violated. For obesity, the graph was crossed (P= 0.03), indicating that the hazard of microvascular complications among patients with obesity and no obesity varied over time.

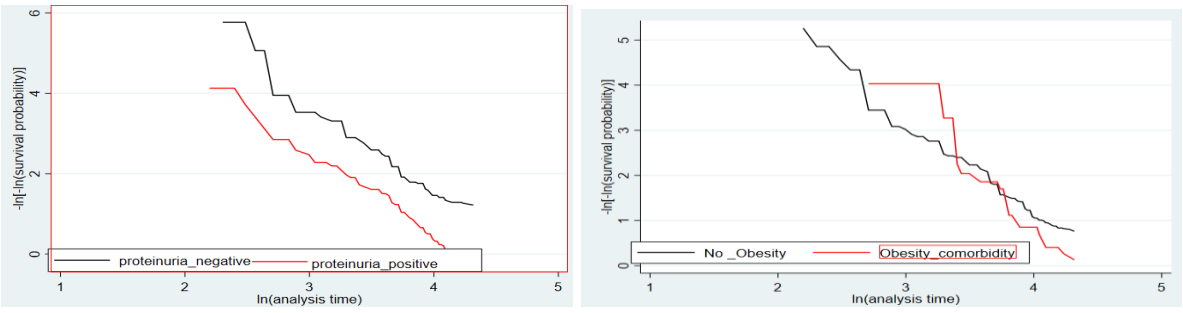

Figure 6: Graphical comparison of Cox-PHA for proteinuria and obesity among T2DM patients in ACSH and MGH, January 2018-March 2024 (n = 447)

Bivariable Cox proportional hazards (Cox-PH) Model

Bivariable Cox-PH regression models were employed to evaluate the impact of each independent variable on the time to any microvascular complications among T2DM patients. Variables that demonstrated a p value of less than or equal to 0.25 in the bivariable analysis were considered for inclusion in the multivariable Cox model. On the basis of the bivariable results, the following variables were considered in the multivariable Cox model: age, weight, BMI, baseline Hgb, systolic HTN, diastolic HTN, baseline FBS, TG level, HDL, LDL, history of HTN, medication type, obesity, anemia and proteinuria.

Model Comparison

The model interaction term added to proteinuria and obesity exhibited the best fit, as indicated by its lowest AIC and BIC values. Compared with the overall model fitness, the model with obesity and proteinuria interacting with time was better fitted, with the smallest value of AIC/BIC (1446.675/1516.375). The model without interaction of time had AIC and BIC values of 1453.428 and 1523.127, respectively.

Predictors of time to onset of Microvascular complications

The results revealed that baseline FBS, TG level, type of medication intake, anemia and proteinuria were significantly associated with time to microvascular complications at the 5% significance level. Baseline FBS was significantly associated with an AHR of 1.007 ($P < 0.001$), indicating that for one unit increased in Baseline FBS, the hazard of microvascular complications was 0.7% more likely keeping other variables constant. Similarly, for TG levels (AHR of 1.003, 95% CI: 1.001, 1.004) for one-unit increased in TG level, the hazard of microvascular complications was 0.3% more likely keeping other variables constant.

The hazard of microvascular complications was 4.7 times higher among Patients on injection alone than patients on single oral agent. Moreover those receiving both injections and oral agents were 4.8 times more at high risk of developing microvascular complications compared to patients on single oral agent (AHR = 4.78, CI; 1.88, 7.70). Additionally, patients taking more than one oral agent had an AHR of 3.8, indicating that they were 3.8 times more likely to

experience microvascular complications than those on a single oral medication, keeping other variables constant.

Additionally, in anemic patients, the hazard of microvascular complications was 58% greater than that in non-anemic patients (AHR = 1.58, CI: 1.01, 2.50). Furthermore, the interaction of proteinuria with time was significantly associated with outcome (AHR = 1.02, 95% CI 1.012, 1.028) meaning that in patients with positive proteinuria, the hazard of microvascular complications 2% more likely over that in patients with negative proteinuria keeping other variables constant.

Table 6: Multivariable Cox-PH model of predictors for time to microvascular complications among T2DM patients in ACSH and MGH, January 2018 to March 2024 (n= 447).

variable	AHR	Standard error	95% CI		P value
			Lower CI	Upper CI	
Baseline FBS	1.007	0.001	1.004	1.009	0.001
TGS level	1.003	0.001	1.001	1.004	0.001
HTN history (Ref = No)					
HTN history Yes	0.855	0.162	0.589	1.239	0.407
Medication (Ref= one oral agent)					
Medication (Injection alone)	4.697	2.721	1.509	14.619	0.008
Medication (Injection+oral)	4.783	1.770	2.315	9.880	0.001
Medication (More than one oral)	3.804	1.368	1.879	7.699	0.001
Stroke (Ref = No)					
Stroke Yes	1.122	0.433	0.528	2.389	0.764
Anemia (Ref = No)					
Anemia (Yes)	1.588	0.366	1.010	2.495	0.045
tvc					
obesity	1.010	0.005	0.996	1.015	0.234
Proteinuria	1.020	0.004	1.012	1.028	0.001

Joint Modeling Analysis

For the joint modeling analysis, longitudinal FBS measurements were analyzed in conjunction with the time to microvascular complications using a Cox proportional hazards model. The mean FBS level was significantly greater among patients who developed microvascular complications than among those who were censored.

As the mean FBS value increased, the risk of microvascular complications also increased, as shown in Figure 7(event). Patients who developed microvascular complications had higher mean FBS levels than those who were censored. In the censored group, the mean FBS level decreased, indicating that at a low level of FBS, no events occurred, as shown in the figure below. Therefore, the joint modeling approach was determined to be more effective than separate analysis in capturing this relationship.

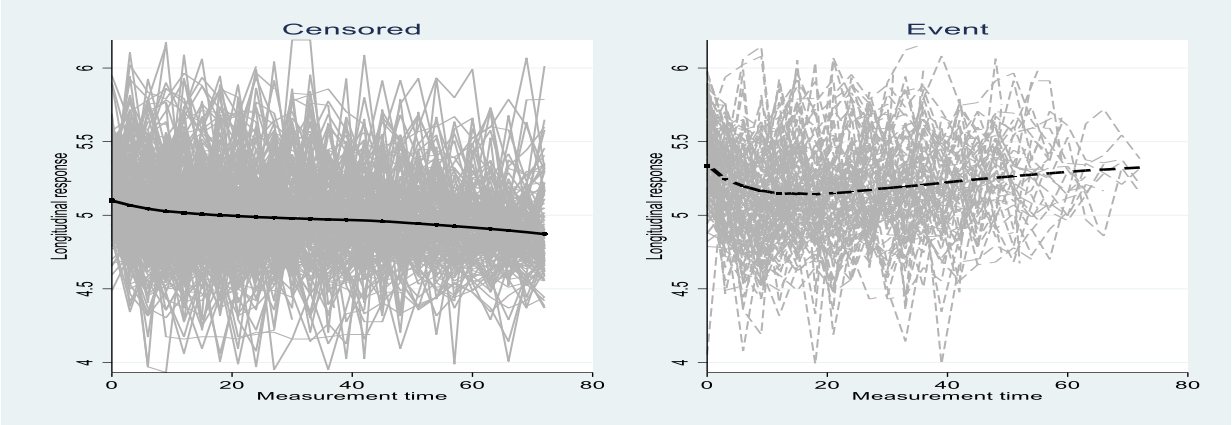

Figure 7: Association between longitudinal FBS and time to microvascular complications among T2DM patients in ACSH and MGH from January 2018 to March 2024 (n = 447)

Multivariable Joint modeling Analysis interpretation

The SD for random intercept was 0.21 indicated; there was considerable heterogeneity in patients' baseline time to and risk of microvascular complications. The SD for the random slope was 0.004, indicated minimal variability in patients' risk of microvascular complications over time. The correlation estimate was -0.609, indicating negative correlation between random intercept and slope. The restricted cubic spline function (individual variation in the FBS over time) had significant effect on the hazard of microvascular complications.

The association parameter, alpha (α value) was 2.94, with a P value <0.001, means that for a unit increase in log(FBS), the hazard of microvascular complications was 18.96 (95% CI: 6.05, 58.56) times higher while keeping the other variables constant.

The longitudinal process from the joint modeling output demonstrated that TG levels, medication types, anemia, and proteinuria were significant determinants of FBS progression. Regarding medication the mean log(FBS) progression among patients on injection plus oral medication was 0.18 mg/dl (95% CI: 0.131, 0.230) higher than that among those who were on a single oral medication. Similarly, the mean of log(FBS) progression among patients taking more than one oral medication was increased by 0.112 (95% CI: 0.070, 0.154). For 1mg/dl increased in TG level, the mean log(FBS) was expected to increase by 0.004 mg/dl (95% CI: 0.003, 0.006). Furthermore, patients who were anemic had a 0.095 mg/dl (95% CI: 0.038, 0.152) higher mean log(FBS) as compared to patients without anemia, while the other parameters remained constant.

The event process of the joint modeling analysis revealed BMI (AHR = 1.061, 95%CI: 0.018, 0.099) indicated that as a patient's BMI increased by one unit, the hazard of microvascular complications was 6% more likely. Additionally when patients TG level increased by one unit the hazard of microvascular complications were 0.3% more likely. In terms of baseline FBS as baseline FBS increased by one unit, the hazard of complications was 0.6% more likely higher (AHR =1.006, 95%CI: 1.003, 1.009).

regarding medication intake, those receiving both injections and oral agents were 2.44 times at high risk of developing microvascular complications compared to patients on single oral agent (AHR =2.44, CI; 0.33, 4.53). Furthermore, patients taking more than one oral agent were 2.57 times more likely to experience microvascular complications than those on a single oral medication (AHR = 2.57, (95% CI: 0.59, 4.53). Furthermore, for patients who were positive for proteinuria, the hazard of complications was 11.3% (95% CI 1.035, 1.197) higher than who were negative to proteinuria holding other variables constant.

Table 7: Joint model analysis of time to microvascular complications associated with FBS measurement among T2DM patients in ACSH and MGH, January 2018-March 2024(n = 447)

Longitudinal process			
Parameters	**Coefficient**	**Std. errors**	**95% CI**
Observe time	-0.0020	0.0001	(-0.003, -0.001***
Time*proteinuria	0.0012	0.0006	(0.0001, 0.0023*
TG level	0.0038	0.001	(0.0014, 0.0062)**
Medication **(**ref =single oral agent)			
Injection medication (IM)	0.163	0.052	(0.061, 0.264)**
Injection and oral medication (IOM)	0.181	0.025	(0.131, 0.230)***
More than one oral medication (MOM)	0.112	0.021	[0.070, 0.154]***
Anemia (yes)	0.095	0.029	(0.038, 0.152)**
Anemia (Ref)			
Hypertension (yes)	-0.039	0.020	(-0.077, 0.0002)
Hypertension (Ref)			
constant	4.899	0.027	(4.846, 4.952)***
Event process			
parameters	**Coefficient**	Standard errors	**95% CI**
Time*Proteinuria (PU)	0.107	0.037	(0.035, 0.180)**
Association(α)	2.940	0.578	(1.808, 4.073)
TG level	0.003	0.001	(0.001, 0.004)**
Baseline FBS	0.006	0.001	(0.003, 0.009)***
medication (ref= single oral agent)			
Injection alone	0.886	0.597	(-0.285, 2.056)
Injection and oral (IOM)	0.898	0.393	(0.127, 1.668)*
More than one oral(MOM)	0.948	0.372	(0.220, 1.677)*
BMI	0.059	0.021	(0.018, 0.099)**
Anemia (yes)	0.154	0.238	(-0.312, 0.620)
_rcs1	1.178	0.196	(0.794, 1.562)
_rcs2	0.013	0.130	(-0.241, 0.267)
_rcs3	0.128	0.088	(-0.044, 0.300)
_cons	-25.42	2.905	(-31.112, -19.725)
SD for random slope	0.004	0.0003	(0.0038, 0.0048)
SD of random intercept	0.210	0.009	(0.193, 0.228)
Corr (time and constants)	-0.609	0.047	(-0.692, -0.509)
SD of residual	0.242	0.002	(0.238, 0.246)

*** P <0.001, ** P <0.01, * P <0.05.

Final Model: The final model was a joint random intercept and slope Cox-PH model and was built from joint model analysis of Cox-PH sub- model and longitudinal sub-model. The model included association parameter, random slope, random intercept, and regression coefficient for independent variables from the joint model. It written as follow from the table below

$$\log(\text{FBSij}) = 4.899 - 0.002 * \text{Time} + 0.0012(\text{Time} * \text{PU}) + 0.004 * \text{TG} + 0.163\text{IM} + 0.181\text{IOM} + 0.112\text{MOM} + 0.095\text{Anemia} + \text{b1i} * \text{Timeij} + \text{b0i} + \varepsilon\text{i}$$

$$\text{hi(t)} = \text{h}_{0(\text{t})}\text{e}^{(0.107\text{PU}+0.003\text{TG}+0.006\text{FBS}_\text{b}+0.059\text{BMI}+0.898\text{IOM}+0.948\text{MOM}+2.94*\log(FBS)}$$

6. DISCUSSION

In this study, survival and longitudinal sub-models were used to identify the determinant factors for the time to develop microvascular complications and changes in the fasting blood sugar level. In addition, the effect of the longitudinal FBS trajectory on the time to microvascular complications was demonstrated using the joint model analysis. The main objective of this study was to predict the associations between longitudinal measurements of FBS and the time to microvascular complications and their predictors among patients with T2DM in ACSH and MGH.

The significant prognostic factors for the hazard of microvascular complications among diabetic patients were BMI, TG level, and baseline FBS, mean FBS progression, type of medication and proteinuria. On the other hand, the rates of change in FBS levels among T2DM patients were significantly determined by time, TG level, type of medication intake, proteinuria and anemia.

The incidence rate of microvascular complications was 6 per 1000 person-months of observation (95% CI: 5–7), which is equivalent to 72 (95% CI: 60–84) per 1000 person-years. This finding was greater than that of a study conducted in Northwest Ethiopia, which reported 21.4 cases of neuropathy per 1000 person-years [26]. This difference could be due to the consideration of diabetic retinopathy and nephropathy in addition to neuropathy. However, it was smaller than findings in Pakistan (IRs of 92.8, 106.2, and 130.2 per 1000 person-year for retinopathy, neuropathy and nephropathy, respectively [14] wolaita Dawro zone (IR = 114 per 100 person-years) [19] and studies at the Felege Hiwot and Debre Markos referral hospitals in Ethiopia (IR: 32.4 per 100 person-years (95% CI: 27.2–37.5) [17]. This discrepancy could be due to differences in the study period and sample size.

A remarkable finding from our study revealed that patients with positive proteinuria had a 12% more likely higher risk of developing microvascular complications (95% CI: 1.04, 1.20) than those without proteinuria. This is in line with studies conducted at Felegehiwot Referral Hospital (AHR = 1.62, CI: 1.08–2.41) and Gondar University (AHR = 1.69, 95% CI: 1.03, 2.78) [20,22]. This finding highlights the role of proteinuria as a critical marker for increased risk of kidney function impairment, systemic inflammation and vascular damage.

In our study, BMI was significantly positively associated with time to microvascular complications (AHR = 1.061, P value 0.005). For one-unit increased BMI, the hazard of microvascular complications was 6% more likely higher. This finding was similar to those of studies performed in Saudi Arabia, Pakistan, and Adama Hospital, Ethiopia[14,15,23]. The possible justification for this could be higher weight leads to chronic hyperglycemia and systemic inflammation, which can damage blood vessels and accelerate the development of microvascular complications.

In this study, baseline FBS levels in diabetic patients were significantly positively associated with time to onset of microvascular complications (AHR =1.006, 95% CI 1.003, 1.009). This was similar findings to studies at Amhara regional state of comprehensive specialized hospitals, Northwest Ethiopia (AHR=2.56) [26]. This study was also aligned with studies at Felegehiwot and Debremarkos referral hospitals, in which the AHR of log FBS was 1.003 [17], and studies in Northwest Ethiopia (AHR = 1.35) [39]. These results highlight the critical role of glucose and lipid metabolism in the progression of microvascular complications among patients with T2DM. Elevated blood glucose levels promote oxidative stress and inflammation, which lead to microvascular complications in T2DM patients. These findings highlight the importance of managing FBS in T2DM patients to potentially mitigate the risk of developing microvascular complications.

Furthermore, the baseline TG level of patients with type 2 diabetes was a significant predictor of time to onset of microvascular complications, with AHR of 1.003 ($P<0.001$). These findings were similar to those of studies conducted at the University of Gonder as well as studies at the Felege Hiwot and Debremarkos referral hospitals and from governmental hospitals in the Harari Region, eastern Ethiopia [22,24,25]. These reports highlight that high levels of TG exacerbate vascular damage in diabetic patients by contributing to endothelial dysfunction, which serves as a precursor to various microvascular complications.

The linear mixed model revealed that patients who had taken more than one oral anti-diabetic medication had an increased mean FBS progression by 11.2% compared with those who were taking only one type of oral medication. Furthermore, those who were received injections and oral medications had increased mean FBS progression by 18.1% compared with those who were

taking only one type of oral medication. Since the outcome variable of FBS in the longitudinal sub-model was converted using log transformation, the percentage increases may not represent exact values. Similarly, the hazard of microvascular complications for patients receiving an injection plus an oral agent or more than one oral agent was 2.455 and 2.581, respectively, greater than that for those receiving a single oral agent. These findings are in line with those of studies conducted at Felegehiwot Hospital and Gonder University and Jimma University Medical Center, Southwest Ethiopia ([17,20,22]. The possible reason for this finding could be that the need for multiple therapies indicates poorer underlying glycemic control and an advanced stage of the disease.

This research also revealed that anemia was significantly positively associated with FBS progression, indicating that anemic patients had higher FBS levels by 7.79% than did those who had no anemia. The lower estimate was due to the log transformation of FBS. This finding contrasts with the literature, which often does not provide an association of anemia with FBS measurements in diabetic patients. The observed associations may be due to several factors. Anemia can lead to reduced oxygen delivery to tissues, potentially affecting metabolic processes and insulin sensitivity. Additionally, chronic inflammation associated with anemia may influence glucose metabolism and contribute to elevated FBS levels [40].

In the joint model analysis, the association parameter (α) was 2.94 (95% CI: 1.808, 4.073). This value indicated the association between longitudinal FBS measurement and time to microvascular complications. This means that for a unit increased in the mean FBS, the hazard of microvascular complications increased by 18.9 times, keeping other variables constant. These showed a strong association between FBS and time to microvascular complications. This result was similar but larger than that of a study conducted in Northwest Ethiopia, which was 1.35 (95% CI: 1.12, 1.63). This discrepancy might be because studies in Northwest Ethiopia focused solely on diabetic retinopathy [25]. Additionally, our results exceed those from studies in Felege Hiwot and Debre Markos referral hospitals, North West Ethiopia, in which α was 0.0126 (95% CI: 0.0150, 0.0102) [17]. This difference could be due to differences in sample size and the irregular availability of diabetes medications during the conflict in the Tigray region.

6.1. Strengths and limitations of the study

The study determined the predictors for time to microvascular complications, the FBS trajectory among T2DM patients and the associations between longitudinal FBS and time to microvascular complications simultaneously. In the joint modeling approach, a robust framework that captures the interconnected nature of these outcomes was used. The use of the Kobo Toolbox tool for data collection further improved the accuracy and efficiency of the study, ensuring reliable and comprehensive data for analysis. Even though, these strengths the study has also some limitations. The study did not consider the Impact of Covid-19 and the Conflict in Tigray region.

7. CONCLUSION AND RECOMENDATIONS

7.1. Conclusion

This study assessed the predictors for time to microvascular complications, the predictors for FBS trajectories and the associations between the FBS trajectory and time to microvascular complications among patients with T2DM. The progression of FBS over time was significantly positively associated with time to microvascular complications, indicating that patients with increasing FBS levels over time had a higher hazard of developing complications. Additionally, the variables baseline FBS, BMI, TG level, proteinuria, and more than one medication were significant predictors of time to microvascular complications among patients with T2DM. Specifically, patients with higher baseline values of FBS, BMI, and TG levels, along with those who were positive for proteinuria and were taking multiple medications, demonstrated a significantly higher hazard of microvascular complications.

This study also assessed the determinant factors for longitudinal FBS progression (change over time) in T2DM patients and revealed that the variables observation time, proteinuria, anemia, more than one medication and TG level were significant determinants of longitudinal FBS progression. These findings highlight the need for close monitoring and targeted management of glycemic control and associated metabolic factors to delay the onset of microvascular complications in patients with T2DM.

7.2. Recommendations

To reduce the risk of T2DM-related microvascular complications, health care providers should implement routine monitoring of glycemic control, including both the FBS level and the glycated hemoglobin (HbA1c) level. Since elevated FBS and HbA1c are associated with an increased risk of microvascular complications, regular assessment can help identify patients needing intensified management. Additionally, patients with positive proteinuria, anemia, increased TG levels, higher BMI and greater FBS progression should receive individualized interventions, which may include medication adjustments, dietary counseling, and consultations with appropriate specialists to delay time to microvascular complications.

Importantly, taking multiple medications, including insulin or combination therapies, often indicates advanced stages of diabetic disease and should prompt treatment review and optimization of the treatment plan rather than being viewed as risky. Furthermore, integrated multidisciplinary care involving nutritionists, nephrologists, and diabetes educators is essential to provide holistic management and improve patient outcomes. Educating patients on the importance of regular monitoring of blood sugar can empower them to participate actively in their care and may delay the time to microvascular complications.

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10. ANNEXES

10.1 Information Sheet

Title of the project: Joint modeling of onset of microvascular complications and longitudinal fasting blood sugar among type 2 diabetes patients at Ayder and Mekelle hospitals, 2025

Name of the principal investigator: Tetemke Mekonen (BSc in public health).

Name of the organization: Mekelle University College of Health Science.

Name of the sponsor: Mekelle University College of Health Science.

Introduction: This information sheet was prepared for governmental health institutes implementing health care services for people. The aim of this form was to clarify the purpose of the research, the data collection procedure, and the permission to conduct the research.

Purpose of the research project:

Understanding the relationship between longitudinal changes in fasting blood sugar and the timing of microvascular complications is essential for improving patient outcomes among diabetic patients. Hence, this research aimed to determine the prognostic factors for both time to microvascular complications and FBS progression over time simultaneously. Moreover, this study assessed the effect of longitudinal FBS on the timing of microvascular complications among T2DM patients using a joint modeling approach.

Methods: To achieve the above stated objective, the study used retrospective longitudinal study among 447 patients in Ayder and Mekelle general hospitals from January 2018 to March 2024. All patients who were free of microvascular complications and had at least two visits were considered for the study, and secondary data from the patients' medical records were collected via the Kobo Toolbox mobile app. A joint linear mixed sub model and Cox-PH was used to analyze the relationship between longitudinal FBS and time to microvascular complications, using STATA software.

Risk and/or discomfort: Since this study was conducted by taking appropriate information from patient medical records, it would not inflict any harm on the patients. The name or any

other identifying information not recorded on the check lists and all the information from the chart were kept strictly confidential and safe. The information retrieved was used for study purposes only.

Benefits: The research had no direct benefit for one whose document /record in this research. But the indirect benefit of the research for the participants and other clients in the program was clear. This is because if program planners were preparing a predicted plan, there was a benefit for clients in the program of receiving appropriate care and treatment services. Overall, the research work has had paramount research benefits for health care planners and managers, especially for chronic kidney disease patients and for the treatment and support of program planning and management.

Confidentiality: To ensure confidentiality, information collected without the names of clients and names was not included in the data collection format. After the data were entered into the computer, they were locked by a password and not disclosed to any other person other than the principal investigator.

Person to contact: This research project was reviewed and approved by the institutional review board of Mekelle University College of Health Science and Ayder Comprehensive Specialized Hospital. If you had any questions, you could contact any of the following individuals (investigator and advisors), and you might ask at any time you want.

Principal investigator: Tetemke Mekonen (BSc) Cell phone +251914890988, Email: tetie20007@gmail.com

- **Advisor**: Mr. Kebede Embaye (Assistant Professor in Biostatistics) Telephone number +251912937551, email: aredom14@gmail.com
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- Mr. Gebretsadkan G. (MPH), email: gere2023@gmail.com

10.2 Data Extraction Sheet

This format was established after reviewing similar literature and guidelines on the management of diabetes mellitus. It is prepared for the collection of socio-demographic factors, clinical factors and comorbidities that are recorded in patients' history sheets or electronic medical

records. Studying longitudinal FBS and the time to microvascular complications among T2DM patients via a joint modeling approach is relevant. The variables were retrieved from patients' medical charts (history sheets) and patients' electronic medical records by qualified health personnel.

8.1 Socio-demographic characteristics

1. Patient medical record number (MRN): _____
2. Sex: Male ☐ Female ☐ unspecified ☐
3. Age: _____

8.2 Clinical factors

1. weight of the patient_____
2. Height of the patient _____
3. Hemoglobin level (mg/dl)_____
4. Blood pressure (BP) at admission
   Systolic BP _____, Diastolic BP:_____
5. Baseline FBS: _____
6. Protein urea: positive ☐ negative ☐
7. If proteinuria is positive, what is the level of proteinuria?_____
8. Triglyceride level:_____
9. High-density lipoprotein level: _____
10. Low-density lipoprotein level: _____
11. Duration of the disease since diagnosis in years:_____
12. Duration of follow-up (months):_____
13. Treatment type: one oral agent☐ more than one oral agent (no insulin)☐, insulin alone or insulin plus oral agents ☐ other: _____

8.3 Baseline measurements of the following comorbidities

1. hypertension: yes ☐ no ☐
2. obesity: yes ☐ no ☐
3. stroke: yes ☐ no ☐
4. Anemia: yes ☐ no ☐

8.4 Patient follow-up status and treatment outcome information were recorded every 3 months during follow-up.

NB: The following variables are measured and recorded from the start of the first visit since recruitment to the study., i.e., month = 0, month = 3, 6,9,12,15,18,21,24,27,30, up to J = number of visits = 72 months)

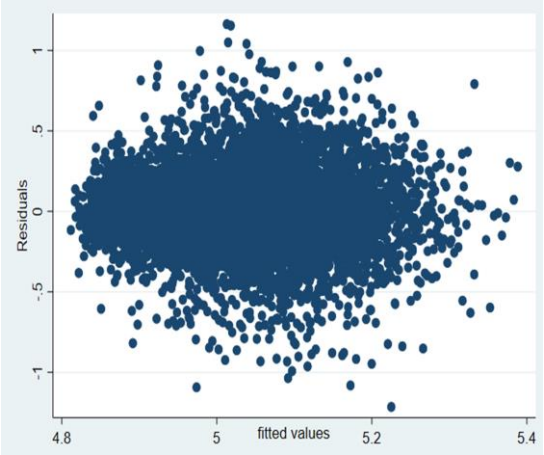
1. Follow-up site: _____
2. Fasting blood sugar level measurement every 3 months starting at J = 0 to J = 72 months)
3. Presence of microvascular complications

    - Retinopathy: yes ☐ no ☐
        - ✓ If yes, date of diagnosis:_____
    - Nephropathy: yes ☐ no ☐
        - ✓ If yes, date of diagnosis <p_____
    - Neuropathy: yes ☐ no ☐
        - ✓ If yes, date of diagnosis <p_____

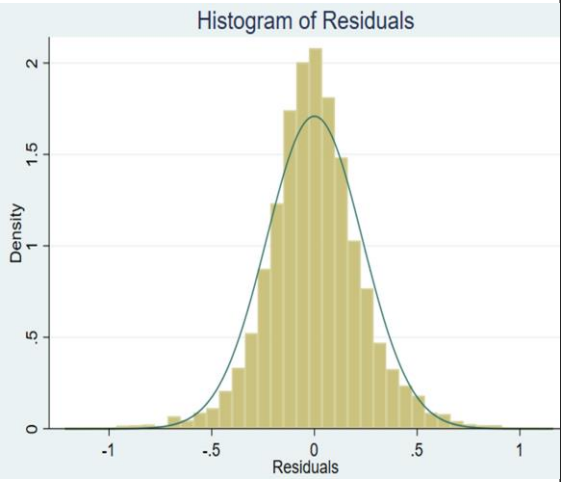
Censored: Lost ☐ Died ☐Referred ☐ Transfer out ☐

10.3: Checking the Adequacy of the Random Effects Assumption

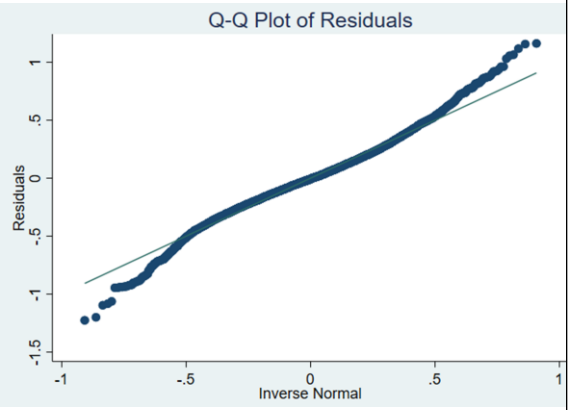
A: residual versus fitted values



B: Histogram of residuals



C: Q–Q plot of residuals



D: summarize residuals

```
. summarize residuals
```

Variable	Obs	Mean	Std. dev.	Min	Max
residuals	7,863	-9.29e-11	.2334298	-1.214873	1.163056

Figure 8: Residual plots for the log FBS of T2DM patients at ACSH and MGH from January 2018 to March 2024 (n = 447)

10.4 Graphical testing for Cox-PHA outputs

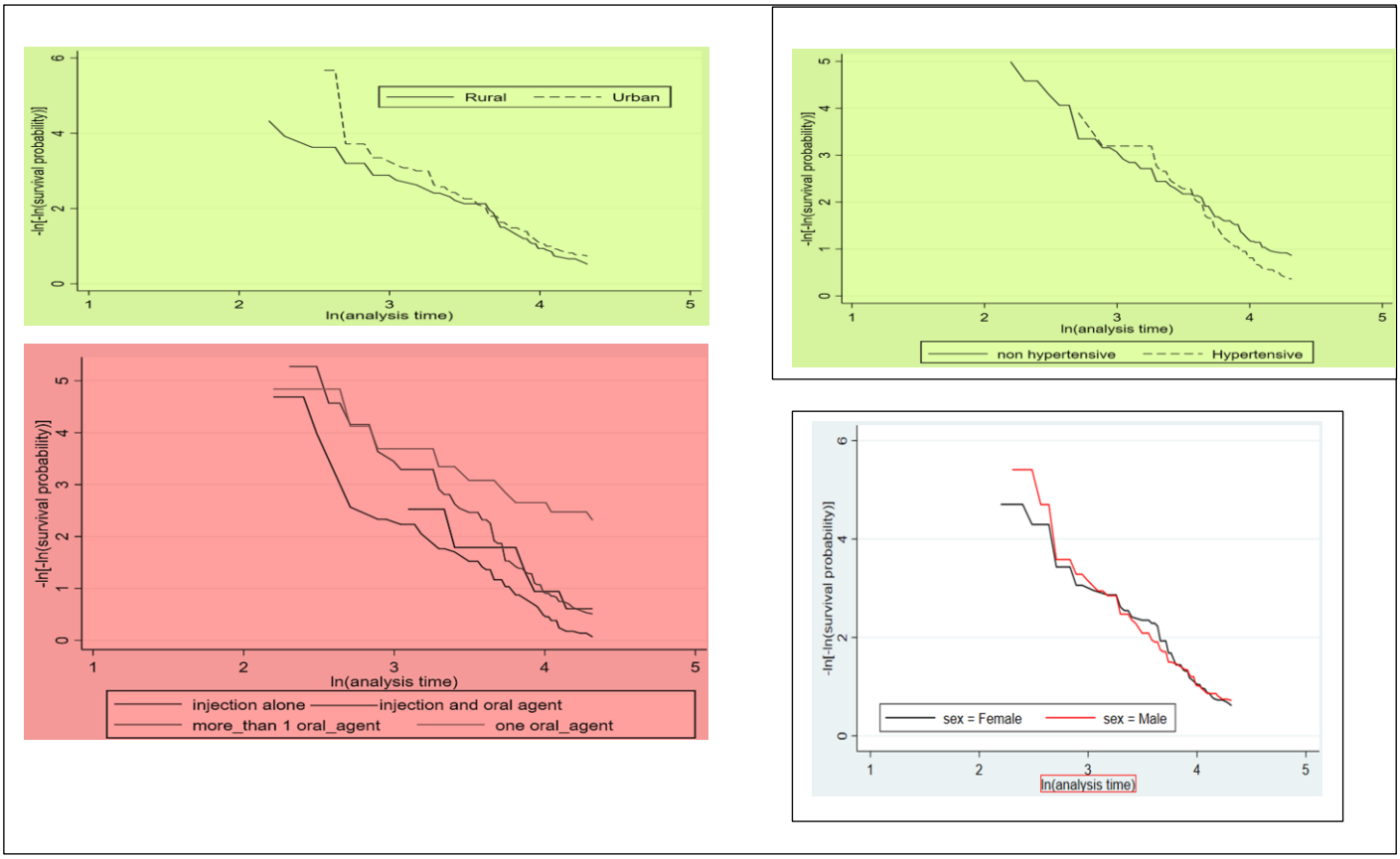


Figure 9 : Graphical checking of PHA among T2DM patients in ACSH and MGH, January 2018-March 2024 (n = 447)

Table 8: Results of the log-rank test for the categorical predictors of T2DM patients at ACSH and MGH, January 2018--March 2024 (n = 447)

Predictors	DF	Chi-square	P value
Sex	1	0.12	0.726
Marital status	3	5.43	0.143
Residence	1	1.15	0.283
HTN	1	6.37	0.012
Medication	3	48.53	0.001
Obesity	1	5.28	0.042
Anemia	1	30.43	0.001
proteinuria	1	67.59	0.001

10.5: Bivariable Cox-PH model analysis output

Table 9: Bivariable analyses of predictors of the time to microvascular complications among T2DM patients at ACSH and MGH from January 2018 to March 2024 (n = 447)

Variable	**HR**	**Standard error**	**z**	**P>|z|**	**[95% confidence interval]**	
age	1.028	0.009	3.27	0.001	1.011	1.046
weight	1.016	0.006	2.60	0.009	1.004	1.029
BMI	1.064	0.018	3.58	0.000	1.029	1.101
Baseline Hgb	0.881	0.039	-2.88	0.004	0.809	0.960
BP systolic	1.012	0.004	3.32	0.001	1.005	1.020
BP diastolic	1.017	0.006	3.12	0.002	1.006	1.029
Baseline FBS	1.009	0.001	8.76	0.000	1.007	1.012
TGs	1.005	0.001	7.40	0.000	1.003	1.006
HDL	0.979	0.007	-3.19	0.001	0.966	0.991
LDL	1.005	0.001	5.71	0.000	1.003	1.007
HTN history yes	1.528	0.260	2.48	0.013	1.093	2.135
medication						
Injection alone	4.976	2.776	2.88	0.004	1.667	14.851
Injection plus oral	8.514	3.058	5.96	0	4.211	17.214
More than one oral	5.227	1.853	4.66	0	2.608	10.473
Obesity yes	1.633	0.355	2.25	.024	1.066	2.5
stroke yes	2.111	0.693	2.28	.023	1.109	4.019
Anemia yes	2.879	0.583	5.22	0.000	1.936	4.282
Protein urea positive	3.666	0.626	7.61	0.000	2.624	5.123

10.6 Data analysis software output

Table 10: A. Missing data analysis output (n = 447)

```
. logistic missing_FBS TGS_level HDL LDL age i.sex_N i.M_status_n i.residence_n weight i.protein_urea_n
note: 1.M_status_n != 0 predicts failure perfectly;
      1.M_status_n omitted and 93 obs not used.

note: 4.M_status_n omitted because of collinearity.

Logistic regression                                     Number of obs =  7,770
                                                        LR chi2(10)   =  33.63
                                                        Prob > chi2   = 0.0002
Log likelihood = -762.78066                             Pseudo R2     = 0.0216
```

missing_FBS	Odds ratio	Std. err.	z	P>|z|	[95% conf.	interval]
TGS_level	.9983695	.0016594	-0.98	0.326	.9951225	1.001627
HDL	.9872724	.0058274	-2.17	0.030	.9759167	.9987602
LDL	.9970871	.001939	-1.50	0.134	.993294	1.000895
age	.9887558	.0087066	-1.28	0.199	.9718376	1.005968
sex_N						
Male	1.549387	.2669591	2.54	0.011	1.105346	2.17181
M_status_n						
divorced	1	(empty)				
married	1.345698	.3878565	1.03	0.303	.7649177	2.367449
single	1.344147	.626467	0.63	0.526	.5391717	3.350939
widowed	1	(omitted)				
residence_n						
urban	.8840051	.158907	-0.69	0.493	.6215047	1.257376
weight	.9708145	.009008	-3.19	0.001	.9533187	.9886313
protein_urea_n						
positive	1.035849	.2036424	0.18	0.858	.7046201	1.522784
_cons	.5698164	.516312	-0.62	0.535	.0964849	3.365199

Table 11: Summary of longitudinally measured FBS using medians and IQRs at every visit (n=447)

```
. tabstat FBS, stat( n median IQR) by( time_point )
Summary for variables: FBS
Group variable: time_point (time point of observation)
```

time_point	N	p50	IQR
0	447	176	76
3	447	157	75
6	447	152	70
9	443	150	62
12	433	148	58
15	416	149	59.5
18	402	148.5	61
21	389	147	61
24	382	152	58
27	365	148	61
30	348	151	67.5
33	335	153	61
36	321	154	66
39	298	147	55
42	280	149	64
45	264	147.5	57.5
48	256	148	58.5
51	232	145	61.5
54	218	143.5	62
57	207	140	48
60	199	138	55
63	189	137	43
66	185	133	37
69	181	129	38
72	179	123	37
Total	7863	148	60

B. RM-ANOMA output

```
. anova FBS time_point id, repeated ( time_point )
```

Number of obs =	7,863	R-squared	=	0.4048	
Root MSE =	44.7392	Adj R-squared	=	0.3670	

Source	Partial SS	df	MS	F	Prob>F
Model	10062699	470	21409.997	10.70	0.0000
time_point	583979.88	24	24332.495	12.16	0.0000
id	9133252.9	446	20478.146	10.23	0.0000
Residual	14795788	7,392	2001.5947		
Total	24858486	7,862	3161.8527		

Link test for linearity assumptions:

```
. predict yhat, xb

. gen yhat2 = yhat^2

. regress FBS_L yhat yhat2
```

Source	SS	df	MS		
Model	81.1838393	2	40.5919197	Number of obs	= 7,863
Residual	715.958475	7,860	.091088865	F(2, 7860)	= 445.63
				Prob > F	= 0.0000
Total	797.142315	7,862	.101391798	R-squared	= 0.1018
				Adj R-squared	= 0.1016
				Root MSE	= .30181

FBS_L	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
yhat	4.865297	2.725677	1.78	0.074	-.4777552	10.20835
yhat2	-.3825123	.269699	-1.42	0.156	-.911194	.1461693
_cons	-9.770244	6.884894	-1.42	0.156	-23.26647	3.725979

Table 12: Multivariate linear mixed model Stata output (n = 447)

```
xtmixed FBS_L time_point ib2.sex TGS_level ib1.HTN_history ib4.medication ib1.Obesity ib1.stroke ib1.Anemia ib1.protein_urea_n || id
: time_point

Computing standard errors:

Mixed-effects ML regression                     Number of obs      =       7,863
Group variable: id                              Number of groups   =         447
                                                Obs per group:
                                                              min =           3
                                                              avg =        17.6
                                                              max =          25
                                                Wald chi2(11)      =      125.28
Log likelihood = -720.97083                     Prob > chi2        =      0.0000
```

time_point	-.0017039	.0002584	-6.59	0.000	-.0022104	-.0011974
sex						
Female	.0304417	.0193318	1.57	0.115	-.0074479	.0683313
TGS_level	.0003705	.0001329	2.79	0.005	.00011	.000631
HTN_history						
yes	-.0439257	.0218124	-2.01	0.044	-.0866771	-.0011742
medication						
injection_alone__insulin_	.1105989	.0572452	1.93	0.053	-.0015996	.2227973
injection_plus_oral_agent	.1645543	.0276528	5.95	0.000	.1103558	.2187528
more_than_one_oral_agent	.1007906	.0236008	4.27	0.000	.0545339	.1470473
Obesity						
yes	.0093445	.0294684	0.32	0.751	-.0484124	.0671015
stroke						
yes	-.0564453	.0508178	-1.11	0.267	-.1560464	.0431557
Anemia						
yes	.0746207	.0316055	2.36	0.018	.0126751	.1365663
protein_urea_n						
positive	.054894	.0228387	2.40	0.016	.0101309	.0996571
_cons	4.920548	.0283219	173.74	0.000	4.865038	4.976058

Random-effects parameters	Estimate	Std. err.	[95% conf.	interval]
id: Independent				
sd(time_point)	.0037274	.0002466	.0032741	.0042434
sd(_cons)	.1810461	.0076621	.1666346	.1967041
sd(Residual)	.242561	.002073	.2385318	.2466583

LR test vs. linear model: chi2(2) = 1900.89 Prob > chi2 = 0.0000

Table 13: Stata output for multivariable Cox regression with time interaction (n = 447)

```
stcox BMI  Baseline_FBS  TGS_level  ib1.HTN_history ib4.medication  ib1.stroke ib1.Anemia, tvc(protein_urea_n ) texp( _t ) strata ( Obesity )

Cox regression with Breslow method for ties

No. of subjects =     447                               Number of obs =   7,863
No. of failures =     140
Time at risk    = 23,556

                                                        LR chi2(10)   = 157.44
Log likelihood = -713.33772                             Prob > chi2   = 0.0000
```

_t	Haz. ratio	Std. err.	z	P>|z|	[95% conf.	interval]
main						
Baseline_FBS	1.006972	.0012958	5.40	0.000	1.004436	1.009515
TGS_level	1.002923	.000797	3.67	0.000	1.001362	1.004486
HTN_history						
yes	.8547697	.1618509	-0.83	0.407	.589758	1.238866
medication						
injection_alone__insulin_	4.697425	2.720926	2.67	0.008	1.509426	14.61867
injection_plus_oral_agent	4.78264	1.770402	4.23	0.000	2.315135	9.880048
more_than_one_oral_agent	3.80374	1.368414	3.71	0.000	1.87926	7.699007
stroke						
yes	1.122807	.4326755	0.30	0.764	.5275858	2.389555
Anemia						
yes	1.587577	.3662308	2.00	0.045	1.010125	2.495138
tvc						
Obesity						
yes	1.00576	.0048553	1.19	0.234	.9962884	1.015321
protein_urea_n						
positive	1.01963	.0041601	4.76	0.000	1.011509	1.027816

```
Akaike's information criterion and Bayesian information criterion
```

Model	N	ll(null)	ll(model)	df	AIC	BIC
.	7,863	-792.0594	-713.3377	10	1446.675	1516.375

```
Note: BIC uses N = number of observations. See [R] BIC note.
```

```
. vif, uncentered
```

Variable	VIF	1/VIF
Baseline_FBS	6.84	0.146116
TGS_level	5.58	0.179111
2.HTN_hist~y	1.75	0.571882
medication		
1	1.11	0.904478
2	1.93	0.518263
3	2.48	0.403734
2.stroke	1.16	0.859351
2.Anemia	1.23	0.810520
2.Obesity	1.26	0.793985
2.protein_~n	1.55	0.643597
Mean VIF	2.49	

```
. corr Baseline_FBS TGS_level HTN_history medication stroke Anemia Obesity protein_urea_n
(obs=7,863)
```

	Baseli~S	TGS_le~l	HTN_hi~y	medica~n	stroke	Anemia	Obesity	protei~n
Baseline_FBS	1.0000							
TGS_level	0.2485	1.0000						
HTN_history	0.1028	0.0565	1.0000					
medication	-0.1722	-0.1169	-0.0916	1.0000				
stroke	-0.0166	0.1465	0.1494	0.0062	1.0000			
Anemia	0.1060	0.1515	0.2227	-0.1002	0.1615	1.0000		
Obesity	0.0834	0.1014	0.1962	0.0437	0.2277	0.0782	1.0000	
protein_ur~n	0.1886	0.2155	0.2327	-0.0916	0.1135	0.2082	0.1031	1.0000

E. Joint modeling Stata output

```
stjm FBS_L TGS_level medication_1 medication_2 medication_3 Anemia_2 protein_urea_level_adj HTN_history2 stroke2 Obesity2 sex1, panel(id) survmo
del(rcs) df(3) rfp(1) timeinterac(protein_urea_n2 protein_urea_n1 ) survcov( TGS_level medication_1 medication_2 medication_3  Baseline_FBS HTN_
Fitting full model:

-> Conducting adaptive Gauss-Hermite quadrature
note: _time_1_protein_urea_n1 omitted because of collinearity.

Iteration 0:   log likelihood = -1399.2105  (not concave)
Iteration 1:   log likelihood = -1398.8231
Iteration 2:   log likelihood =  -1398.671
Iteration 3:   log likelihood = -1398.6709

Joint model estimates                             Number of obs      =      7863
Panel variable: id                                Number of panels   =       447
                                                  Number of failures =       140
Log-likelihood = -1398.6709
```

	Coefficient	Std. err.	z	P>|z|	[95% conf.	interval]
Longitudinal						
_time_1	-.0019054	.0003121	-6.10	0.000	-.0025171	-.0012936
_time_1_pr~2	.0013695	.0005837	2.35	0.019	.0002255	.0025134
_time_1_pr~1	0	(omitted)				
TGS_level	.0003941	.0001199	3.29	0.001	.0001591	.0006291
medication_1	.1625342	.0518205	3.14	0.002	.0609678	.2641005
medication_2	.1806615	.0252173	7.16	0.000	.1312365	.2300865
medication_3	.1122658	.0213686	5.25	0.000	.0703841	.1541476
Anemia_2	.0950204	.028914	3.29	0.001	.0383499	.1516909
protein_ur~j	.0156069	.0083321	1.87	0.061	-.0007238	.0319375
HTN_history2	-.0385466	.0197057	-1.96	0.050	-.0771691	.0000759
stroke2	-.0192306	.0465161	-0.41	0.679	-.1104005	.0719394
Obesity2	.0244238	.026939	0.91	0.365	-.0283757	.0772233
sex1	.0343572	.0175136	1.96	0.050	.0000312	.0686831
_cons	4.898764	.0269726	181.62	0.000	4.845898	4.951629
Survival						
assoc:value						
protein_ur~n	.107385	.0369551	2.91	0.004	.0349544	.1798155
_cons	2.94044	.5778146	5.09	0.000	1.807945	4.072936
xb						
TGS_level	.0027294	.000876	3.12	0.002	.0010126	.0044463
medication_1	.8858522	.5972673	1.48	0.138	-.2847702	2.056475
medication_2	.8976985	.3931801	2.28	0.022	.1270796	1.668317
medication_3	.9480745	.3717283	2.55	0.011	.2195004	1.676649
Baseline_FBS	.0060433	.0013547	4.46	0.000	.0033881	.0086986
HTN_history2	-.0867891	.1906998	-0.46	0.649	-.4605538	.2869757
Anemia2	.1541945	.2378464	0.65	0.517	-.3119758	.6203648
BMI	.0585053	.0209158	2.80	0.005	.0175111	.0994995
stroke2	.0907186	.3852725	0.24	0.814	-.6644016	.8458388
_rcs1	1.1779	.1959068	6.01	0.000	.7939302	1.561871
_rcs2	.0131591	.1295903	0.10	0.919	-.2408331	.2671514
_rcs3	.1280372	.0875258	1.46	0.144	-.0435102	.2995846
_cons	-25.4188	2.904983	-8.75	0.000	-31.11246	-19.72514

Random effects Parameters	Estimate	Std. Err.	[95% Conf.	Interval]
id: Unstructured				
sd(_time_1)	.0042311	.0002498	.0037688	.0047502
sd(_cons)	.2099567	.0089071	.1932052	.2281606
corr(_time_1,_cons)	-.608588	.0467101	-.6921551	-.5089194
sd(Residual)	.2415131	.0020414	.2375449	.2455476

Longitudinal submodel: Linear mixed effects model
Survival submodel: Restricted cubic spline hazard model
Integration method: Adaptive Gauss-Hermite quadrature using 5 nodes
Cumulative hazard: Gauss-Kronrod quadrature using 15 nodes
Akaike's information criterion and Bayesian information criterion

Model	N	ll(null)	ll(model)	df	AIC	BIC
.	7,863	.	-1400.423	31	2862.846	3078.914

Note: BIC uses N = number of observations. See [R] BIC note.

JM for random Intercept Model

```
. stjm FBS_L TGS_level medication_1 medication_2 medication_3 Anemia_2  HTN_history2 stroke2 Obesity2 sex1, panel(id) survmodel(rcs) d
> f(3) rfp(0) timeinterac(protein_urea_n2 protein_urea_n1 ) survcov( TGS_level medication_1 medication_2 medication_3  Baseline_FBS HT
> N_history2 Anemia2 BMI stroke2 ) assoccov(protein_urea_n)
Joint model estimates                                 Number of obs.      =      7863
Panel variable: id                                    Number of panels    =       447
                                                      Number of failures  =       140

Log-likelihood = -1442.6726
```

	Coefficient	Std. err.	z	P>|z|	[95% conf.	interval]
Longitudinal						
_time_1	-.0485224	.0045158	-10.74	0.000	-.0573733	-.0396716
_time_1_pr~2	.0208039	.0061161	3.40	0.001	.0088167	.0327912
_time_1_pr~1	0	(omitted)				
TGS_level	.0004091	.0001191	3.44	0.001	.0001757	.0006424
medication_1	.1426619	.0517786	2.76	0.006	.0411777	.2441461
medication_2	.1798101	.0249007	7.22	0.000	.1310056	.2286147
medication_3	.11416	.0212664	5.37	0.000	.0724787	.1558414
Anemia_2	.0887804	.028556	3.11	0.002	.0328117	.1447492
HTN_history2	-.0351591	.0195119	-1.80	0.072	-.0734018	.0030835
stroke2	-.029882	.0460049	-0.65	0.516	-.1200499	.0602858
Obesity2	.0174709	.0267035	0.65	0.513	-.034867	.0698088
sex1	.0348578	.0174643	2.00	0.046	.0006285	.0690872
_cons	4.987627	.0280975	177.51	0.000	4.932557	5.042697

Survival assoc:value						
protein_ur~n	.1199449	.0370996	3.23	0.001	.0472311	.1926587
_cons	2.025679	.5788536	3.50	0.000	.8911473	3.160212
xb						
TGS_level	.0028062	.0008494	3.30	0.001	.0011414	.0044709
medication_1	1.119867	.5951726	1.88	0.060	-.0466495	2.286384
medication_2	1.113149	.3923798	2.84	0.005	.3440991	1.8822
medication_3	1.097496	.3695785	2.97	0.003	.3731355	1.821856
Baseline_FBS	.006336	.0013411	4.72	0.000	.0037075	.0089645
HTN_history2	-.1022881	.1881421	-0.54	0.587	-.4710397	.2664636
Anemia2	.2633954	.2336084	1.13	0.260	-.1944687	.7212594
BMI	.0609655	.0204476	2.98	0.003	.020889	.101042
stroke2	.1538305	.3793629	0.41	0.685	-.5897071	.8973681
_rcs1	1.158897	.192448	6.02	0.000	.7817063	1.536089
_rcs2	.0387034	.1274779	0.30	0.761	-.2111487	.2885555
_rcs3	.1457578	.0866109	1.68	0.092	-.0239964	.3155121
_cons	-21.09044	2.892584	-7.29	0.000	-26.7598	-15.42108

Random effects Parameters	Estimate	Std. Err.	[95% Conf.	Interval]
id: Unstructured				
sd(_time_1)	.0640888	.0039799	.0567443	.0723838
sd(_cons)	.2304145	.0124319	.2072925	.2561157
corr(_time_1,_cons)	-.6856765	.0393138	-.7552977	-.6007526
sd(Residual)	.2437219	.0020651	.2397078	.2478032

Model	N	ll(null)	ll(model)	df	AIC	BIC
.	7,863	.	-1442.673	31	2947.345	3163.413

1, summery of follow up period

```
. sum Duration_of_followup, detail

                    Duration_of_followup
-------------------------------------------------------------
      Percentiles      Smallest
 1%           12              6
 5%           24              6
10%           30              6       Obs                7,863
25%           46              6       Sum of wgt.        7,863

50%           72                      Mean            58.59494
                      Largest         Std. dev.       18.01397
75%           72             72
90%           72             72       Variance          324.503
95%           72             72       Skewness       -1.032819
99%           72             72       Kurtosis        2.793898
```