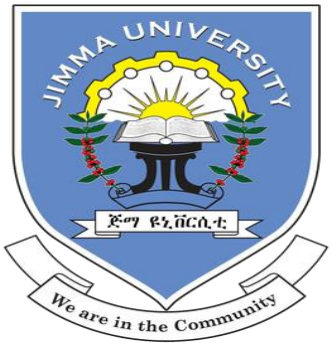

JIMMA UNIVERSITY

INSTITUTE OF HEALTH

FACULTY OF MEDICAL SCIENCE

DEPARTMENT OF BIOMEDICAL SCIENCE

Assessment of Cardiac Autonomic Neuropathy and Associated Factors Among Type 2 Diabetes Mellitus Patients at Arsi University Referral and Teaching Hospital, Southeast Ethiopia; A Cross-Sectional Study

By: Abdulmalik Jeben (BSc)

Thesis Submitted to Department of Biomedical Sciences, Faculty of Medicine Institute of Health, Jimma University in Partial Fulfillment of the Requirements for the Degree of Master of Science (MSc) in Medical Physiology

March 2022

Jimma, Ethiopia

Assessment of Cardiac Autonomic Neuropathy and Associated Factors Among Type 2 Diabetes Mellitus Patients at Arsi University Referral and Teaching Hospital, Southeast Ethiopia: A Cross-Sectional Study

By: Abdulmalik Jeben (BSc)

Advisors:

1. Mr. Teshome Gobena (Ass. Prof. of Medical Physiology)

2. Mr. Moyeta Beriso (Msc, Lecturer of Medical Physiology)

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ABSTRACT

Background: Cardiovascular Autonomic Neuropathy is one of the most underdiagnosed complication of diabetes mellitus. It is the impairment of autonomic control of cardiovascular system. Due to a significant risk of cardiac arrhythmias, silent myocardial ischemia, and sudden death, patients with CAN have a five-fold increased mortality risk. Currently, there is no published data in Ethiopia on the prevalence of cardiac autonomic neuropathy and associated factors amongst type 2 diabetic patients.

Objective: The present study aimed to assess cardiac autonomic neuropathy and associated factors among Type 2 diabetes mellitus patients at Arsi University Referral and Teaching Hospital, Asella, Ethiopia 2021.

Methods: Hospital-based cross-sectional study design was conducted among Type 2 diabetes mellitus patients who were under follow-up care in Arsi University Referral and Teaching Hospital. Systematic random sampling technique was used and data were collected by using semi constructed questionnaire, reviewing patient cards, measuring anthropometry and vital sign of patients. Cardiovascular Autonomic Neuropathy was assessed using the five autonomic function tests: resting heart rate, heart rate response to deep breathing, heart response to standing from supine, change in systolic blood pressure during standing, and in diastolic blood pressure during sustained handgrip. The data were entered into Epi data version 4.6.0.2 and transported to SPSS version- 26 software for further analysis. Multi-variant logistic regression was used to identify independent predictors of Cardiovascular Autonomic Neuropathy; $P < 0.05$ was considered statistically significant.

Results: Among 260 subjects, 131 were suffering from CAN giving an overall prevalence of CAN 50.4% 95% CI (44.6-56.2). From all the CAN patients, 68 (26.2%) CI (21.2-31.5) had moderate CAN (Ewing score 2) while 63(24.2%) CI (18.8-29.2) had severe CAN (Ewing scores >2). Older age [AOR = 4.106, 95% CI: (2.182 - 7.725), P<0.01], longer duration of diabetes [AOR = 2.324, 95% CI: (1.281 - 4.216), P < 0.05], P < 0.05], poor glycemic control [AOR = 2.287, 95% CI: (1.146 - 4.562)), P < 0.05], presence of hypertension [AOR = 1.980, 95% CI (1.108 - 3.540)),and retinopathy [AOR = 2.024, 95% CI (1.15 2-3.556), P < 0.05] were significant risk factors for CAN in T2DM patients.

Conclusion and Recommendation: . CAN accounts more than half of T2DM and associated with Older age, being hypertensive, longer duration of diabetes, poor glycemic control, and having retinopathy which may indicate that Clinicians in the DM OPD should be aware of the high prevalence of CAN and Consider CAN screening in all diabetes patients during their regular follow-up.

Keywords: - Diabetic cardiac autonomic neuropathy, cardiac autonomic neuropathy

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LIST OF ACRONYMS AND ABBREVIATION

ANS	Autonomic nervous system
AURTH	Arsi University Referral and Teaching Hospital
BMI	Body mass index
CAN	Cardiac autonomic neuropathy
CARTs	Cardiovascular autonomic reflex testing
CKD	chronic kidney disease
DAN	Diabetic autonomic neuropathy
DBP	Diastolic blood pressure
DCAN	Diabetic cardiac autonomic neuropathy
DM	Diabetes mellitus
ECG	Electrocardiogram
FBG	Fasting blood glucose
IDF	International Federation of diabetes
IL	Interleukin
IR	Insulin resistance
OS	Oxidative stress
ROS	Reactive oxygen species
SBP	Systolic blood pressure
T2DM	Type 2 diabetes mellitus
TNF	Tumor necrosis factor

CHAPTER 1: INTRODUCTION

1.1. Background

The autonomic nerve system is responsible for maintaining physiological homeostasis and controlling acute stress responses. The sympathetic nervous system has a favorable effect by releasing norepinephrine, which causes a rise in heart rate, conduction velocity, and contractility. Increased parasympathetic tone, which is mediated by acetylcholine, has a negative chronotropic, dromotropic, and inotropic effect (1,2).

The resting heart rate in men is reduced to roughly 50–70 beats per minute (bpm) from its intrinsic range of 110–120 bpm by the baseline vagal ‘tone.' Vagal control is manifested in respiratory sinus arrhythmia with reduced and prolonged cardiac durations during inspiration and expiration, respectively, especially when observed under undisturbed stable conditions without physical activity and/or respiratory rate and tidal volume fluctuations (3).

Afferent neural impulses are transferred from the heart to the intrinsic neurons of the heart, to extracardiac intrathoracic ganglia (e.g., stellate ganglion), to the spinal cord, and to the brain stem to accomplish autonomic regulation of the heart. These afferent neural signals are processed by various areas of the nervous system to regulate the sympathetic and parasympathetic nerves' cardio motor neural output to the heart.This autonomic nervous system control of the heart is can be disturbed by disorders that affect the autonomic nerves directly, such as diabetes mellitus and primary autonomic failure syndromes (4–6).

Diabetes mellitus (DM) is a metabolic and vascular condition caused by abnormalities in insulin synthesis, action, or both; and defined by persistent hyperglycemia and alterations in carbohydrate, lipid, and protein metabolism (7).

One of the most prevalent effects of diabetes is neuropathy, which affects 50% of all persons with the disease. Neuropathy can be polyneuropathy, mononeuropthy, or autonomic neuropathy. Diabetic autonomic neuropathy (DAN) is a sneaky consequence of diabetes that develops slowly over time and silently robs diabetic people of their ability to detect hypoglycemia or a heart attack (8).

Diabetic neuropathies are the most common long-term consequences of diabetes, and they are a broad set of disorders that affect many sections of the nervous system and affects the circulatory, gastrointestinal, genitourinary, and integumentary systems, the most common of which is cardiac autonomic neuropathy (CAN) (9,10).

CAN is one of the most underdiagnosed diabetic disorders and a key risk factor for CVD in diabetics. Type 2 diabetes mellitus (T2DM) has a higher prevalence of cardiac autonomic neuropathy (CAN) than type 1 diabetic Mellitus (T1DM) (11,12).

After all other causes have been ruled out, diabetic cardiac autonomic neuropathy (DCAN) is defined as a loss of autonomic regulation of the cardiovascular system in people with diabetes(13,14).

Diabetes-related CAN affects autonomic nerve fibers that innervate the heart and blood vessels, resulting in heart rate and vascular dynamics anomalies such as resting tachycardia, exercise intolerance, orthostatic hypotension, and silent myocardial ischemia (12).

CAN symptoms and signs should be assessed at the time of diagnosis in people with T2DM, especially if they have poor glycemic control (HbA1c > 7%), have a major cardiovascular disease risk factor, or have other chronic DM problems by Ewing test is the gold standard approach for assessing CAN, which includes a battery of five cardiovascular autonomic reflex tests and asses both sympathetic and parasympathetic nervous systems (15,16).

Based on the results of cardiovascular autonomic reflex tests (CARTs), the diagnosis can be early/possible CAN (1 abnormal cardiovagal test), definite/ confirmed CAN (2 abnormal cardiovagal tests), and severe/advanced CAN (2. abnormal cardiovagal tests with orthostatic hypotension) (17).

Toronto Consensus Panel on Diabetic Neuropathy recommends that diagnosis of CAN should be based on the use of cardiovascular autonomic reflex (Ewing) tests that is, heart rate response to deep breathing, standing up from sitting, and the Valsalva maneuver and blood pressure response to standing up or handgrip (18).

Heart rate response to deep breathing (expiration: inspiration ratio and E : I ratio) and HR response to standing (30 : 15 ratio) which is the ratio of the longest R-R interval (between the 20th and 40th beat) to the shortest interval (between the 5th and 25th beat) elicited by a position change from horizontal to vertical measure parasympathetic function mainly the ability of the vagal nerve to slow the HR during procedures which increase HR. The HR response during the Valsalva maneuver represents also parasympathetic activity, but autonomic changes also include a sympathetic component. The BP response to standing and the BP response to sustained handgrip caused by the muscle contraction using a handgrip dynamometer show changes in sympathetic function and involve baroreflex-mediated blood presser fluctuations (19).

1.2. Statement of problem

According to the International Diabetes Federation (IDF) in 2021 globally diabetes was estimated to affect 537 million adults (20-79 years of age) , or more than 1 in 10, they predicted 643 million people will have diabetes by 2030 (11.3% of the population) and the number will jump to a staggering 783 million (12.2%) by 2045; 24 million adults (20-79 years of age) were living with diabetes in Africa and this figure is estimated to increase to 33 million by 2030 and 55 million by 2045; In Ethiopia 1.9 million or 3.3% of adults (20-79 years of age) were diabetic (20).

Diabetes hurts people's functional abilities and quality of life, resulting in severe morbidity and mortality (12) and called the primary health-care challenge of the twenty-first century (11).

In 2021 diabetes directly caused 6.7 million deaths world wide and 416,000 deaths in Africa Region(21). According to the study done in Germany during a 9-year follow-up, 10.5% of the nondiabetic and 30.6% of the diabetic population decreased (22).

Diabetes and its complications cause personal unhappiness and place a financial strain on society's resources (23). Nearly 10% of the global health expenditure is spent on diabetes care, which is equal to USD760 billion in 2019, and this is expected to reach USD845 billion by the year 2045 (24). In Ethiopia, the cost of treating diabetes and preventing complications among adults (20–79 years) was projected to be between $ 80 and $200 million in 2015. Understanding the frequency of type 2 DM complications, as well as identifying associated risk factors, will be crucial for better disease management and prevention (25).

The cardiovascular disorders are the leading cause of morbidity and mortality in DM accounting for more than 60% of the mortality rate among people with DM. Of those cardiovascular disorders CAN is a significant consequence eventhough it is often neglected, misunderstood, and under-evaluated (11,26–28).

CAN is a serious chronic complication of diabetes and an independent predictor of cardiovascular disease mortality. It causes abnormalities in heart rate as well as blood pressure control. It is very difficult to detect DCAN at early stages because it stays silent for several years, that is why it is often over looked during both in diagnosis and treatment of diabetes (7).

The prevalence of confirmed CAN is around 20% and increases up to 65% with age and diabetes duration (29). The study conducted in Uganda reveal a high prevalence of CAN among individuals in routine outpatient care for diabetes mellitus 52.2% (29.4% early CAN while 20.4% and 2.3% were definite and severe (advanced) CAN respectively) and older age, longer duration of diabetes and coexistence of retinopathy are associated with CAN (24). The Canadian cohort study has demonstrated high prevalence rates for CAN (44%), which continued to rise throughout the study(23). The study conducted in China showed that the rate of CAN+ was significantly higher in the diabetic group than the control group by using the Ewing test (44.4%), heart rate variability analysis (44.4%), or heart rate turbulence analysis (52.22%) (30).

CAN is linked to an increased risk of death and morbidity (31). Due to a significant risk of cardiac arrhythmias, silent myocardial ischemia, and sudden death, patients with CAN have a five-fold increased mortality risk(22). According to a retrospective cohort study conducted in Hungary T2DM persons with CAN at baseline had a 31% increased hazard of mortality compared to participants without CAN (32). Over a 5- to 10-year period, estimates of mortality related to cardiovascular autonomic neuropathy range from 27% to 56% (16).

Due to the increased frequency of T2DM and limited information on the prevalence of CAN and associated risk factors in Sub-Saharan Africa including Ethiopia it is critical to examine DCAN prevalence and associated factors, as this information would aid the afflicted population and stakeholders in tackling the problem. So this study aimed to assess cardiac autonomic neuropathy and associated factors among type 2 diabetes mellitus patients at AURTH DM centre.

1.3. Significance of the study

Findings from the present study will help to identify the magnitude and pattern of CAN among T2DM patients and some associated factors. The finding of this study might be helpful for policy makers and health planners to develop preventive, early detection, and intervention policies based on the prevalence of diabetic autonomic neuropathy and its associated factors. The diabetic Mellitus population as a whole may benefit from this research in terms of seeking medical guidance, self-management, and understanding the impact of cardiac autonomic neuropathy on their health. Furthermore, the study can serve as baseline data and contribute significantly to the addition of vital information for academics interested in further research in this field.

CHAPTER 2: LITERATURE REVIEW

2.1 An Over View of Cardiac Autonomic Neuropathy Among T2DM

The development and progression of neurological and cardiovascular disorders are linked to diabetes and a state of chronic insulin resistance (33). The pathophysiology is complex and poorly understood. Reduced capillary blood flow, basement membrane thickening, and the production of microaneurysms are considered to be caused by oxidative stress, inflammation, and the buildup of advanced glycation end products (34).

Insulin resistance is linked to increased expression of inflammatory factors such as tumor necrosis factor-a and interleukin-6. Insulin signaling is disrupted by inflammatory cytokines, which leads to insulin resistance. Insulin resistance may thus have a role in the DCAN process through a variety of methods. Glycation end products, activation of poly (ADP ribose) polymerase reductase pathways, direct DNA damage, negative effects on neural regeneration and repair, decreased neurotransmitter release and synapse function, altered Na/K/ATPase pump, and endoplasmic reticulum damage (35).

Diabetes damages autonomic nerves in a length-dependent manner, similar to somatic neuropathies. Damage to the vagus nerve induces resting tachycardia and a decrease in parasympathetic tone, accounting for about three-quarters of parasympathetic activity. As a result, CAN frequently begin in the vagus nerve, the body's longest parasympathetic autonomic nerve, and the source of nearly three-quarters of parasympathetic activity; damage to the vagus nerve induces resting tachycardia and a drop in total parasympathetic tone (36). As the condition progresses, sympathetic denervation causes heart rate and blood pressure to become refractory to sleep, exercise, stress, or chemical stimulus such as adenosine (37).

2.2. The magnitude of Cardiac Autonomic Neuropathy Among T2DM

The incidence of Cardiac Autonomic Neuropathy (CAN) in diabetics was 16 %, (38), 22.1%(28), 30.7 %(39), 64.2 % (40), 53.2%(41),and 58%(42) according to studies conducted in India. Other studies in India discovered an overall prevalence of CAN in the T2DM population of 80.39% (Early CAN34.31 %, definite CAN33.33 %, and moderate CAN 9.80 %)(18,43–45),and 70% (early CAN 25%, definite CAN 24%, and severe CAN 21% (46), and 90 (early in 20%, definitive in 45% and advanced in 35%)(22).

According to a studies conducted in China, the prevalence of CAN in T2DM was 62.6%(47), 25.1% (48), 34.67% (definite CAN 31.23% and severe CAN 3.44%) (49), 41.8% (14),and 39.9% (63).

According to a study conducted in Nigeria, 51 out of 176 diabetic participants (29%) had CAN (50). Based on at least one or more abnormal cardiovascular autonomic reflex tests, researchers in Uganda discovered a prevalence of CAN of 52.2 percent (95 percent CI 46.3–58.0 percent) (51). The prevalence of cardiac autonomic neuropathy, defined as at least one abnormal test, was 39.7% (123 310 individuals) in research conducted in France (33). Cardiac autonomic neuropathy was found in 51 of the 138 persons tested (37.0 percent in a Peruvian investigation (52). In a study conducted in Pakistan, the prevalence of CAN was determined to be 40%, with early, definite, and severe involvement identified in 13.9%, 12.5%, and 13.9 percent, respectively (53).

A cross-sectional study conducted in Bangladesh on 62 patients with type 2 diabetes mellitus having electro physiologically diagnosed peripheral neuropathy showed that all patients had CAN 14.52% had early, 26.67% had definitive and 59.68% had severe CAN (54).

According to the study conducted in Romania, the prevalence of CAN was 39.1% in T2DM and 61.8% in T1DM patients and, poor glycemic control and the long duration of diabetes were the key risk factors for developing CAN in either T1DM or T2DM (55).

2.3. Diabetic Cardiac Autonomic Neuropathy and Sociodemographic Characteristics

CAN dysfunction was equally prevalent in both genders in the research population in south India, but there was a statistically insignificant female dominance. Males aged 51-60 years (90 percent) and females aged 61-70 years were the most impacted age groups (100 percent) (56). Even though men had a larger frequency of CAN than women, the differences were not statistically significant, according to a study conducted in Italy to determine the prevalence of CAN in patients with newly diagnosed type 2 diabetes. According to a South Korean study in terms of the presence of CAN, there were no significant differences in age, sex, HbA1c level, or diabetes duration (57).

An Indian study discovered that patients with CAN were older ($P = 0.0005$) than patients without CAN (58). According to the American study there is no difference in age (35).

According to a Chinese study, the occurrence of CAN in diabetic patients is connected to the duration of the condition and the patient's age (59). Physiological aging is associated with an impairment of ANS structure and function and reduced BDNF levels are responsible, at least in part, for these phenomena(60). According to the Australian study, the early CAN group

was older (P.001)(7). The prevalence of CAN increases with age and diabetes duration, according to a study conducted in Germany (13). According to the American study conducted by the American Diabetes Association the prevalence of verified CAN, based on at least two abnormal heart rate test results, ranges from 16.6% to 20% and rises to 65 percent as people get older and their diabetes lasts longer (28).

There were no significant variations in genotypes between individuals with CAN and those who did not, according to an Iraqi study (33).

2.4. Diabetic cardiac autonomic neuropathy and characteristics of T2DM

In a cohort study conducted in Denmark, the prevalence of manifest CAN was 9.0 percent at the 6-year follow-up and 15.1 percent at the 13-year follow-up, with an annual incidence of 1.8 percent (61).

In a Ugandan study of ambulatory diabetic patients, CAN was shown to be more common in those who had diabetes for more than ten years and those who had diabetic retinopathy(52).

According to the study conducted in south India in patients with type 2 diabetes, neuropathy ($p=0.006$) and retinopathy ($p=0.03$) were found to be strongly linked with CAN (62).

According to a Brazilian study in both type 1 and type 2 diabetes mellitus (DM) patients, diabetic retinopathy has been linked to cardiac autonomic dysfunction (54).

According to a study conducted in India, CAN is linked to the prevalence of microvascular sequelae such as peripheral neuropathy, nephropathy, and retinopathy (63).

The Chinese study backs up the idea that HbA1c variability is substantially linked to the presence and severity of CAN in patients with long-term type 2 diabetes (64). According to a separate study carried out in India, poor glycemic management and obesity are linked to the occurrence of CAN (65). A study conducted in Iran discovered no link between CAN and glycemic control levels (62). The study conducted in Taiwan showed that nephropathy, duration of diabetes, blood pressure, uric acid, and the presence of retinopathy and metabolic syndrome significantly correlated with the CASS score(66). The study conducted in Pakistan also revealed that Cardiac autonomic dysfunctions are common in poorly controlled type 2 diabetes(67). The study conducted in Switzerland also showed that CAN is associated strongly with poor glycemic control ($p\text{-value} < 0.05$)(35).

A study conducted in Ukraine found that having a long history of DM I and DM II, poor glycemic control, and coexistence of CAN and peripheral neuropathy were all substantially associated with a high prevalence of CAN (33). The American Diabetes Association found that rigorous hyperglycemia and blood pressure therapies had significant protective benefits on CAN, with average risk reductions of 16 and 25 percent, respectively, after a median follow-up of 5 years (68). According to a study conducted in India Cardiac autonomic neuropathy is associated with the length and glycemic control of diabetes, the existence of peripheral neuropathy, and an early morning blood pressure increase, but not with the patient's age or sex (52).

According to the study conducted in Romania, poor glycemic control and the long duration of diabetes were the key risk factors for developing CAN in either T1DM or T2DM (46).

Hyperglycemia, obesity, and hypertriglyceridemia are all negatively connected to CAN indicators, according to data from a Danish study, however, these effects fade over time (69). According to another study conducted in Denmark Patients with CAN were older and had a longer duration of diabetes, higher systolic blood pressure, more nephropathy and retinopathy, and a higher vibration threshold (70). A modest collection of factors, including HbA1c, hypertension, distal symmetrical polyneuropathy (DSP), and retinopathy, predict the risk of CAN, according to a study conducted in the United Kingdom (71).

The Australian study found that a greater BMI or central adiposity, poor glycemic control, and a higher total daily insulin dose throughout adolescence and early adulthood predict cardiac autonomic dysfunction (72).

2.5. Diabetic cardiac autonomic neuropathy and substance use

According to an Egyptian study, chewing Khat enhances insulin resistance by increasing resistin levels and cathinone-induced catecholamine release, resulting in higher FBG, PBG, HOMA-IR, cortisol, copper, calcium, and lower zinc and insulin levels in type 2 diabetes individuals (73).

Chronic, heavy drinking has a deleterious effect on the autonomic nervous system, according to research conducted at Yale University School of Medicine in the United States of America, and maybe a sensitive biomarker of craving and relapse (74). According to the study conducted in Qatar CAN was present in 15.3% of the participants. Hypertension, smoking, antihypertensive use, body mass index, dyslipidemia, and albuminuria were significantly higher in participants with CAN than those without CAN ($p<0.05$ (5).

2.6. Diabetic cardiac autonomic neuropathy and comorbidities

The study conducted in Greece among type II DM demonstrated that the odds of CAN increased with higher waist circumference, systolic blood pressure, hypertension, smoking, diabetes duration, fasting glucose, HbA1c, LDL cholesterol, triglycerides, retinopathy, peripheral neuropathy, glomerular filtration, and microalbuminuria (75).

According to an Italian study, patients with confirmed CAN had a considerably higher BMI. Patients with CAN were also more likely to be treated for hypertension, according to the researchers (28). A study conducted in India discovered a link between CAN and diabetic renal disease(76). According to an Indian study, microalbuminuria and the progression of renal illness in adults are linked by cardiovascular autonomic neuropathy (77).

Confirmed or severe cardiac autonomic neuropathy, regardless of the type of diabetes, age, BMI, or HbA1c, was found to be a risk factor for hypertension in a French investigation(78). According to a Taiwanese study, CAN was independently related to CKD in diabetic individuals, with an adjusted odds ratio of 2.77 (95 percent CI, 1.15–6.68). With increasing CKD severity in diabetes, there was a positive linear trend in the probabilities of CAN (79).

According to the findings of a study conducted in South Korea, 65 of the 100 individuals examined had CAN and 26 had diastolic dysfunction. Furthermore, 19 diabetic individuals with diastolic dysfunction (73.1%) had CAN complications. Diastolic dysfunction was more common in diabetic patients with CAN than in diabetic patients without CAN (29.2% vs 20.0%) and CAN was more common in diabetic patients with diastolic dysfunction than in diabetic patients without diastolic dysfunction (73.1 percent vs 62.2 percent, p0.05) (80).

A study conducted in China and Canada discovered a link between CAN and the severity of coronary stenosis in patients with type 2 diabetes mellitus and coronary artery disease (CAD) (coronary artery diseases) (81). According to a study conducted in Nigeria, the prevalence of DCAN was 26.9%, and hypertension and serum creatinine were independent predictors of CAN. According to the findings of a Chinese study, TG and the severity of lipid profile are both strongly and independently linked with DCAN (82).

According to an Indian study, Both HRR and HRV recovery were impaired in DM and HTN. Moreover, the co-existence of HTN had a synergistic effect, causing further worsening of autonomic recovery in T2DM (83). There essential role of vascular factors in the development of neuropathy(62).

2.7. Conceptual framework

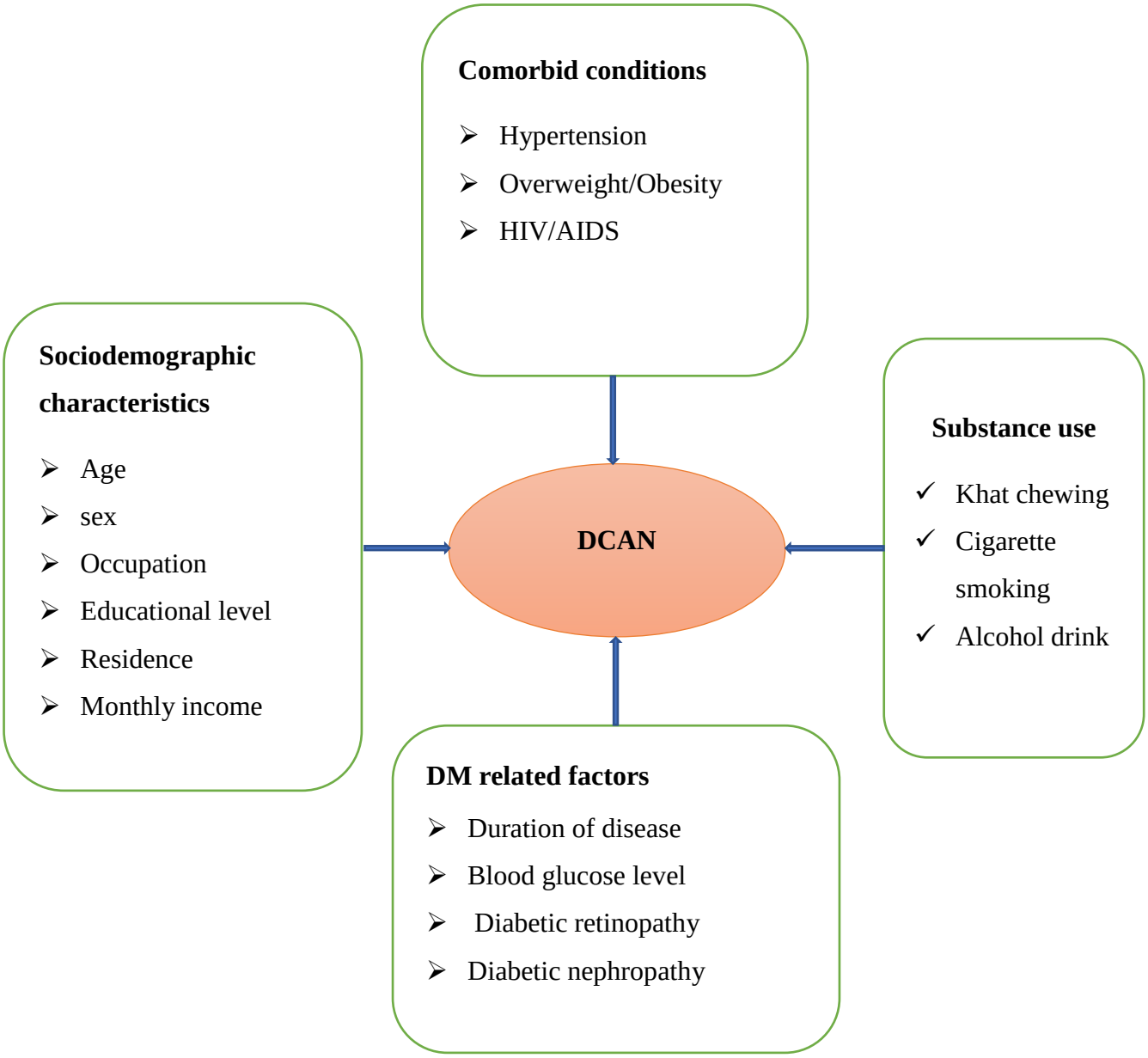

Figure 1 Adapted schematic presentation (conceptual framework) showing potential risk factors of cardiac autonomic neuropathy among type 2 DM patients AURTH, Arsi, Ethiopia, 2021

CHAPTER 3: OBJECTIVES

3.1 General objective

To assess cardiac autonomic neuropathy and associated factors among T2DM patients at Arsi University Referral and Teaching Hospital, Asella, Ethiopia 2021

3.2 Specific objectives are:

- To determine the magnitude of cardiac autonomic neuropathy among T2DM patients
- To describe the severity pattern of cardiac autonomic neuropathy among T2DM patients
- To assess factors associated with cardiac autonomic neuropathy among T2DM patients

CHAPTER 4: MATERIALS AND METHODS

4.1 Study area and period

The research was conducted at Arsi University Referral and Teaching Hospital's DM center in Asella town, Ethiopia, which is located 175 kilometers southeast of Addis Ababa. AURTH provides services to almost 3.5 million people in the catchment area, including chronic care follow-up for diabetes and other diseases. Aside from providing care, it also serves as a learning environment for medical and health science students to further develop their professional knowledge and skills. There are 2000 T2DM patients on follow up at this hospital during data collection perid. This study was conducted from October 1 through November 30, 2021.

4.2 Study design

The study was conducted using an institution-based cross-sectional study design.

4.3 Population

4.3.1 Source population

All T2DM patients had regular follow-up at the diabetic center of AURTH.

4.3.2 Study population

All selected T2DM patients who were on follow-up at the diabetes center of AURTH during the study period freely give consent while meeting the inclusion criteria.

4.4 Eligibility criteria

4.4.1 Inclusion criteria

All T2DM patients aged ≥18 years on follow-up who came to Arsi University Referral and Teaching Hospital's diabetic center during the period of data collection and those who had at least 2 previous consecutive fasting blood glucose records.

4.4.2 Exclusion criteria

Exclusion criteria were the presence of: Other diseases associated with autonomic nervous system affection like thyroid disease. Severe systemic diseases (cardiac, pulmonary, renal, and malignancy), and an acute sickness within the last 48 hours. Underlying cardiac illnesses like coronary artery disease, ischemic heart disease, rheumatic heart disease, arrhythmia, and cardiac failure. Smoking and drinking of caffeine or alcohol-containing beverages 2 hours

before the time of data collection. Uncooperative and physically disabled patients; Pregnant and breastfeeding women were also excluded from the study.

4.5 Sample size determination and sampling technique

4.5.1 Sample size determination

The sample size was determined by using single population formula $n=((z1\_(\alpha/2))^2 p(1-P))/d^2$ for estimating single population proportion based on the following assumptions: Where n* = sample size without considering the finite population correction factor, **Z** α /2 = Z statistic for a level of confidence (95%) = **1.96,** P = expected prevalence or proportion, =22.7% (**0.227)** which was taken from study of Prevalence and Correlates of Cardiovascular Autonomic Neuropathy Among Patients with Diabetes in Uganda (52), 1-P=q= 0.773 (negative prevalence), and d = the marginal error = 0.05 so **n*=** $((1.96)^2\ 0.227\ (0.773))/(0.05)^2 = 270$. After addition of 10% for non-response rate and using the correction formula (nf=n/(1+n/N)) where N = total population of T2dm patients the final sample size was 260.

4.5.2 Sampling technique

A systematic random sample technique was used for T2DM patients. The center's overall number of type 2 diabetes patients was 2000. The total number of patients was divided by the sample size (260) to compute the constant, which was 7, and number 1 was chosen from the ten numbers using the lottery technique. Then, starting with the first patient, every seventh visitor was recruited.

4.5.3 Data collection procedure

Three trained BSc Nurses collected the data and another senior nurse served as supervisor. The principal investigator also participated in the data collection process as a supervisor. Primarily fulfillment of the conditions listed as inclusion criteria was checked and the presence of the conditions listed as exclusion criteria was ruled out by objective assessment of individuals.

Data on socio-demographic, economic, behavioral characteristics, and symptoms of diabetic neuropathy were collected using semi-structured interviewer-administered questionnaires.

Clinical data such as duration of diabetes, previous glycemic control, treatment type, comorbidities, and complications of diabetes) obtained from the patient's medical record.

Physical measurements such as weight, height, BMI, heart rate, and blood pressure were taken using standardized methods and adjusted equipment. Weight was measured by digital scale (Seca Germany) with light cloths and no wearing of shoes to the near closest 0.1 in kilogram. The scale was calibrated to zero before and after each measurement. Height was measured in centimeter using a stadiometer at the Frankfurt plane; participants stood in an erect posture with their shoulders level hand at their side, thighs and hells comfortable together, buttocks, scapula, and head positioned in contact with the vertical stand of the stadiometer with no shoes wearing and the records were taken to the nearest 0.5 centimeter. Body mass index (BMI) was calculated as weight divided by height squared (kg/m2) and Values of BMI was classified as follows: BMI ≤ 18.5 Kg/m2 underweight, BMI = 18.5-24.9 Kg/m2 normal weight, BMI = 25-29.9 Kg/m2 overweight and BMI ≥ 30 Kg/m2 obese. Blood Pressure was measured by qualified BSc nurses using the multi-parameter electrocardiography (ECG) patient monitor (Mindray – MEC – 1200, china 2010) from the upper arm after a patient sat for at least 5 minutes. The hand was at heart level to minimize the gravity effect during blood pressure measurement. And all the anthropometric and BP measurements were measured twice.

The average of three conscetive fasting blood glucose (FBG) level (two from the patents records and the last done during the day of data collection) was used to determine glycemic control level. The last FBG test was done after inserting a test strip into glucometer and pricking the side of patients fingertip with the needle (lancet) provided with the test kit and touching and holding the edge of the test strip to the drop of blood and blood sugar level displayed on a screen of the glucometer after a few seconds was recorded as the last fasting blood glucose level.

All participants have undergone a battery of five autonomic function tests, with each test receiving a score of 0–1 (84). Five standard Ewing non-invasive tests with multi-parameter electrocardiography (ECG) patient monitor (Mindray – MEC – 1200, china 2010) were used to assess cardiac autonomic functioning. All patients were thoroughly instructed on specific exercises such as hand gripping, standing, and deep breathing (59). Resting heart rate, heart rate response to deep breathing, immediate heart rate (HR) reaction to standing, orthostatic hypotension (OH), and hand gripping test (HRT) were all examined on the enrolled patients (85).

Resting Heart Rate (RHR): Heart rate was taken while the patients were in the supine position and RHRs of more than 110 beats per minute were deemed abnormal (86).

Heart Rate (HR) Response to Deep Breathing Test: The participants were asked to do deep breath for one minute at 6 breaths per minute with five seconds for inspiration and five seconds for expiration. The heart rate (HR) in beats per minute was calculated during each cycle of inspiration and expiration. The mean value of the difference in heart rate during inspiration and expiration (Maximum HR- Minimum HR) was calculated(87).

Immediate Heart Rate (HR) Response to Standing: After getting the basal resting heart rate, the participant was asked to stand up from a supine position and heart rate was measured again. The 30th second and 15th-second heart rate after standing was used to calculate the 30:15 ratio(88).

Blood Pressure Response to Standing: The patient's blood pressure (BP) was measured first in the supine position, and then the patient was told to stand up. After 2 minutes of standing, blood pressure was taken again. A drop in systolic blood pressure of more than 20mmHg or a drop in diastolic blood pressure of more than 10mmHg was considered unhealthy (89).

Hand Gripping Test (HGT): The patient's blood pressure was taken in the supine position, and then he or she was instructed to squeeze a little ball in his or her hand for about 5 minutes while lying on the bed, after which his or her blood pressure was taken again. A rise in diastolic blood pressure of fewer than 15 millimeters of mercury (mmHg) was considered unhealthy (90).

RHR was considered normal when its value was < 100bpm, borderline when 100-110bpm and abnormal when it was >110bpm; the value of immediate HR response to standing was considered normal when it was 1.04 and abnormal when it was 1.00; the value of BP response to standing was considered normal at 10 and abnormal at 20, and the value of BP response to sustained handgrip was considered normal at 16 and abnormal at 10.

Each of these five tests was given a score of 0 for normal, 0.5 for borderline, and 1 for abnormal results, and the total of these five tests was used to calculate the Ewing score, which was used to determine the severity of CAN (91).

As a result, parasympathetic neuropathy was diagnosed in people who have two abnormal HR-based tests. To diagnose sympathetic neuropathy, at least one aberrant test result from two blood pressure-based assays must be present (92).

In this study, patients were diagnosed as normal or no CAN (no abnormal result in all tests), early or possible CAN (one abnormal result in any test), definite CAN(2 abnormal results in heart rate-based tests), or severe CAN (2 abnormal results in heart rate-based tests and at least one abnormal result in blood pressure-based tests). The first two groups (no CAN and early CAN) were categorized as CAN negative while the last two groups (definite CAN and severe CAN) were categorized as CAN positive (22).

4.6 Study variables

4.6.1 Dependent variable:

DCAN:(No CAN, CAN (Definite CAN or Severe CAN))

4.6.2 Independent variables:

Sociodemographic variables: - Age, gender, monthly income, marital status, occupation, ethnicity, religion, educational status, and place of residence.

Clinical characteristics of T2DM: Glycemic control, treatment type drug adherence, duration of diabetes from diagnosis, and the occurrence of other microvascular complications (retinopathy and nephropathy).

Substance use: Khat chewing, cigarette smoking, and alcoholic consumption)

Comorbidities: Hypertension (Bp), obesity (BMI), obstructive sleep apnea, and HIV infection.

4.7 Operational definition

No CAN: all five tests are normal or borderline

Early or possible CAN: - one abnormal result in any test

DCAN: – aberrant results in two heart rate-based Ewing tests (heart rate response to deep breathing and immediate heart rate response to standing).

Severe DCAN: - aberrant results in two heart rate-based Ewing tests (heart rate response to deep breathing and immediate heart rate response to standing) plus at least one aberrant result in blood pressure-based tests (blood pressure response to standing and diastolic blood pressure response to sustained handgrip).

Good glycemic control: - FBS 70 - 126 mg/dl on three-month average.

Poor glycemic control: FBS > 126 mg/dl on a three-month average

Substance use – use of at least one of the drugs on purpose (alcohol, Khat, cigarettes)

Current user- Anyone who has used any substance at least once in the last 30 days

Ever use- Use of any of the substances at least once in an individual's lifetime.

Hypertension- A person with a systolic blood pressure of 140mmHg or higher and/or a diastolic blood pressure of 90mmHg or higher, or on antihypertensive medication.

Body mass index (BMI): weight in kg to the square of height in m^2 will be used to classify as

Underweight- A person having a BMI of <18.5Kg/m2

Normal – A person having a BMI of 18.5_ 24.95Kg/m2

Overweight- A person having a BMI of 25 _ 29.9Kg/m2

Obese- A person having a BMI of ≥30Kg/m2

4.8 Data analysis procedure

The data were double-checked for accuracy before being entered into Epi Data version 4.6.0.2 and exported to SPSS version 26.0 for further analysis. For categorical data, frequency and percentage were utilized, whereas, for continuous data, mean and standard deviation were employed. Binary logistic regression was used to investigate the crude relationship between each variable and DCAN. Multiple logistic regression was considered for variables with a p-value of less than 0.25 in binary logistic regression. The independent factors related to DCAN were identified using a multiple logistic regression model with the backward likelihood ratio method. Multiple logistic regression revealed that exposure variables with a p-value less than 0.05 and a 95 percent confidence interval were substantially linked with DCAN. Finally, the model's fitness was tested using the Hosmer and Lemeshow Test, with the final model receiving a P 0.328for DCAN and indicating that it was good.

4.9 Data quality management

The pre-test was done on 5%of sample size out of the study area which was at Bekoji Hospital and further modification was done in the questionnaires accordingly. Two days training was given for data collectors on the study's goal, interviewing methodology, measuring techniques, and ethical considerations before collecting data. Two senior nurses with experience on ECG patient monitoring machines were served as data collectors and another senior nurse served as supervisor. The questionnaires were translated into Afan Oromo and Amharic before being retranslated into English by another person to ensure

uniformity. The questionnaires were verified daily by the supervisor and primary investigator for consistency and completeness.

4.10 Ethical consideration

Ethical clearance was obtained from Jimma University's Institute of Health review board, and a letter of collaboration was asked and obtained from AURTH medical director's office. To begin data collection, study participants were given their informed consent. Full measures to minimize infection transmission and COVID 19 prevention protocols were strictly followed as recommended by WHO. All incidental findings identified during screening were reported to responsible physicians for additional care.

4.11 Dissemination plan of results

After the study is completed, the findings will be defended at Jimma University's Department of Biomedical Sciences, Medical Physiology Course Unit Institute of Health, and submitted to Jimma University's School of Graduate Studies, Biomedical Sciences Department, AURTH, and other relevant bodies. Workshops, seminars, and a publication in an internationally reputable journal will also be used to disseminate the findings.

CHAPTER 5: RESULTS

5.1 Socio-Demographic Characteristics of the Study Participants

A total of 260 T2DM patients were enrolled in this study, with a 100% response rate, 95 (36.5%) of whom were female; 88(33.8) of study participants were above 60 years old(lower age was 30 years old and maximum age was 100 years old); 222 (85.4%) of study participants were married, and 65 (25%) did not have a formal education; 159 people (61.2%) were from the urban;129 (49.6%) of the people were orthodox Christians; whereas 169 (65%) were Oromo; 48 (18.5%) of the participants earns less than 2000ETB per month while 46 (17.7%) of study participants were civil servants, and Mean age of study participants was 56.87±13.709 years old. (**Table 1**)

Table 1 Socio-demographic characteristics of the study participants (n = 260) at AURTH, Asella, Ethiopia, 2021.

Variable	Category	Frequency	Percent
Age	> 60	88	33.8
	</= 60	172	66.2
Gender	Male	165	63.5
	Female	95	36.5
Residence	Urban	159	61
	Rural	101	39
Education status	Collage or above	53	20.4
	Primary or secondary school	142	54.6
	No formal education	65	25.0
Marital status	Single	38	14.6
	Married	222	85.4
Religion	Orthodox	129	49.6
	Muslim	116	44.6
	Others	15	5.8
Ethnicity	Oromo	169	65.0
	Amhara	70	26.9
	Others	21	8.1
Occupation	Non-government employee	214	82.3
	Government employee	46	17.7
Level of income	<2000/month	48	18.5
	>/=2000/month	212	81.5

Single = Unmarried, Divorced, Widowed; Others = Protestant, Wakefata, Gurage, Silte, Tigre; Non-Government Employee = Private, Housewife, Farmer, Merchants, Daily Labor

5.2 Clinical, comorbidity, and behavioral characteristics of the study participants

127 (48.8%) patients were on oral hypoglycemic agents (OHA), only,103 (39.6%) were on insulin only as first-line therapy, and 30 (11.5%) were on both OHA and insulin, 158(60.8%) of the study participants said that they take their anti-diabetic drugs regularly; In terms of co-morbidities, During the study period, 112 (43.1%) of the patients were hypertensive; Systolic blood pressure was greater than or equal to 140mmhg in 83 (31.9percent), while diastolic blood pressure was greater than or equal to 90mmhg in 45 (17.3percent); Nephropathy and retinopathy were seen in 91 (35.0%) and 122 (46.9%), respectively, whereas obstructive apnea symptoms were reported in 108 (41.5%); 88 (33.8%) had been diabetic for more than 10 years; The majority of the study subjects 199 (76.5%) had FBG levels higher than 126 mg/dl;83 (33.5%) of them had BMI of (>24.9); and 25.0%, 11.9%, and 19.6% respectively had a history of alcohol consumption, cigarette smoking, and Khat chewing. **(Table2)**

The mean (Std. Deviation) duration of diabetes, BMI, and FBG were 8.52(5.934) years, 23.6395(3.85840) kg/m^{2}and 186.4831(63.26605) g/dl respectively. **(Figure 2)**

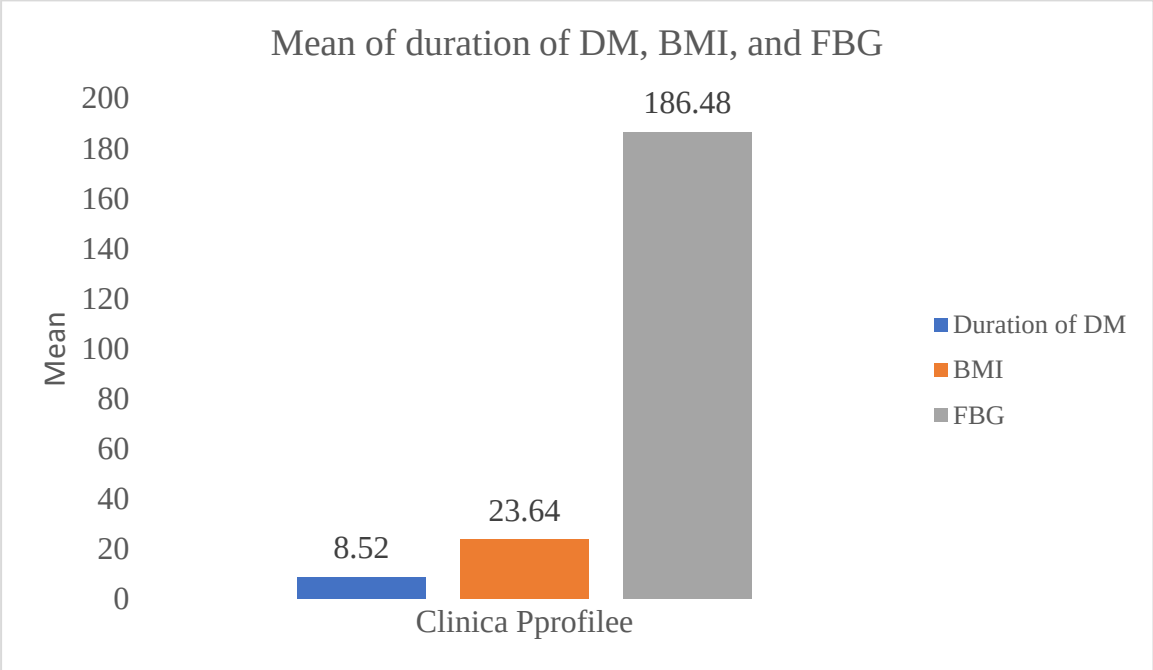

Figure 2. Graphical presentation of Mean of duration of dm, BMI, and FBG (n = 260) at AURTH, Asella, Ethiopia, 2021.

Table 2 Clinical profile of the study participants (n = 260) at AURTH, Asella, Ethiopia, 2021.

Variable	Category	Frequency	Percent
Type of treatment	Insulin only Insulin + OHA OHA only	103 30 127	39.6 11.5 48.8
Regularly taking antidiabetics	No Yes	102 158	39.2 60.8
Hypertension	Yes No	112 148	43.1 56.9
Nephropathy	Yes No	91 169	35.0 65.0
Retinopathy	Yes No	122 138	46.9 53.1
Obstructive sleep apnea symptoms	Yes No	108 152	41.5 58.5
Systolic blood pressure In mmhg	>/=140 <140	83 177	31.9 68.1
Diastolic blood pressure	= />90 <90	45 215	17.3 82.7
Duration of diabetes in years	>10 </=10	88 172	33.8 66.2
BMI	>/=25 <25	87 173	33.5 66.5
FBG in mg/dl	>126 70 - 26	199 61	76.5 23.5
Resting heart rate bpm	≥100 <100	61 199	23.5 76.5
Alcohol	Yes No	65 195	25.0 75.0
Smoking	Yes No	31 229	11.9 88.1
Khat chewing status	Yes No	51 209	19.6 80.4

5.3 Common symptoms of CAN among study participants

Weakness, dizziness, palpitations, up-body-pers, and chest pain were among the most common symptoms they reported, whereas 19 (7.3 percent) said they were unaware of diabetes consequences such as hyperglycemia and hypoglycemia. **(Table 3)**

Table 3 Common symptoms of CAN among study participants at AURTH, Asella, Ethiopia, 2021

Variable	Category	Frequency	Percent
weakness	No	68	26.2
	Yes	192	73.8
Dizziness	No	105	40.4
	Yes	155	59.6
Upper body perspiration	No	149	57.3
	Yes	111	42.7
Palpitations	No	148	56.9
	Yes	112	43.1
Chest pain	No	117	45.0
	Yes	143	55.0
Awareness of complications	No	19	7.3
	Yes	241	92.7
Frequent urination	No	66	25.4
	Yes	194	74.6
Nocturia	No	81	31.2
	Yes	179	68.8
Syncope	No	157	60.4
	Yes	103	39.6
Dyspnea	No	133	51.2
	Yes	127	48.8

5.4 Cardiac autonomic reflex tests for diagnosis of cardiac autonomic neuropathy

In Ewing tests for parasympathetic autonomic neuropathy, 47(18.1%) had borderline values and 11(4.2%) of patients had abnormal values for resting heart rate; 148(56.9%) and 59(22.7%) participants had abnormal and borderline values for heart rate response to deep breathing, respectively; while 140(53.8%) and 59(22.7%) had abnormal and borderline values for heart rate response to the immediate standing test, respectively; In Ewing tests for sympathetic autonomic neuropathy, abnormal and borderline values for blood pressure response to standing were found in 56(21.5 percent) and 111(42.7%), respectively, while abnormal and borderline values for diastolic blood pressure response to sustained handgrip were found in 108(41.5 percent) and 124(47.7%). **(Table 4)**

Table 4 Ewing test of the study participants (n = 260) at AURTH, Asella, Ethiopia, 2021.

Variable	Category	Frequency	Percent	95% Confidence Interval	
				Lower	Upper
Resting heart rate	Normal	202	77.7	72.3	83.1
	Borderline	47	18.1	13.1	23.1
	Abnormal	11	4.2	1.9	6.9
Hr-response to deep breathing	Normal	53	20.4	15.8	25.8
	borderline	59	22.7	17.7	27.7
	abnormal	148	56.9	51.5	62.7
The standing 30:15 ratio	Normal	61	23.5	18.5	28.5
	Borderline	59	22.7	17.7	27.7
	Abnormal	140	53.8	48.1	60.0
Bp response to standing	Normal	93	35.8	30.0	41.5
	Borderline	111	42.7	36.5	49.2
	Abnormal	56	21.5	16.5	26.5
Bp to sustained handgrip	Normal	28	10.8	7.3	14.6
	Borderline	124	47.7	41.5	53.1
	Abnormal	108	41.5	35.8	47.3

5.5 Prevalence of CAN among study participants

CAN was found in 131 of the 260 patients studied, resulting in an overall prevalence of 50.4 % (95% CI) (44.6-56.2). **(Figure 3)**

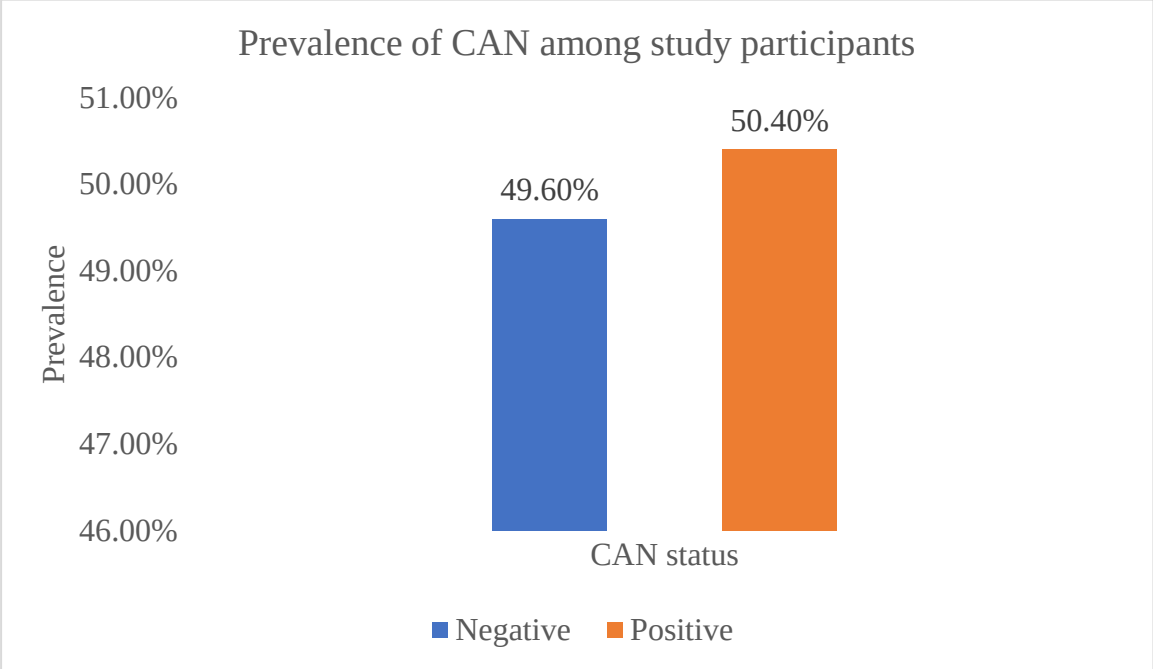

Figure 3. Prevalence of CAN among study participants (n = 260) at AURTH, Asella, Ethiopia, 2021.

5.6 Severity Pattern of CAN Among Study Participants

The prevalence of possible (early)CAN (Ewing score 1 or 1 abnormal result in any test) was 29.2(76/260) [(95% CI) (23.8-34.6)].

26.2 percent (68/260) of the patients had moderate CAN (Ewing score 2 or 2 abnormal results in heart rate based testa) [95% CI (21.2-31.9)].

24.2 percent (63/260) of the patients had severe CAN (Ewing scores >2 or at least 2 abnormal heart rate based tests and 1 abnormal blood pressure based test) [95%CI (18.5-29.6)].

Only 20.4% (53/260) of the patients had no abnormality in cardiac autonomic function tests and were so categorized as having no CAN [95%CI (15.8-25.4)]. **Figure 4**

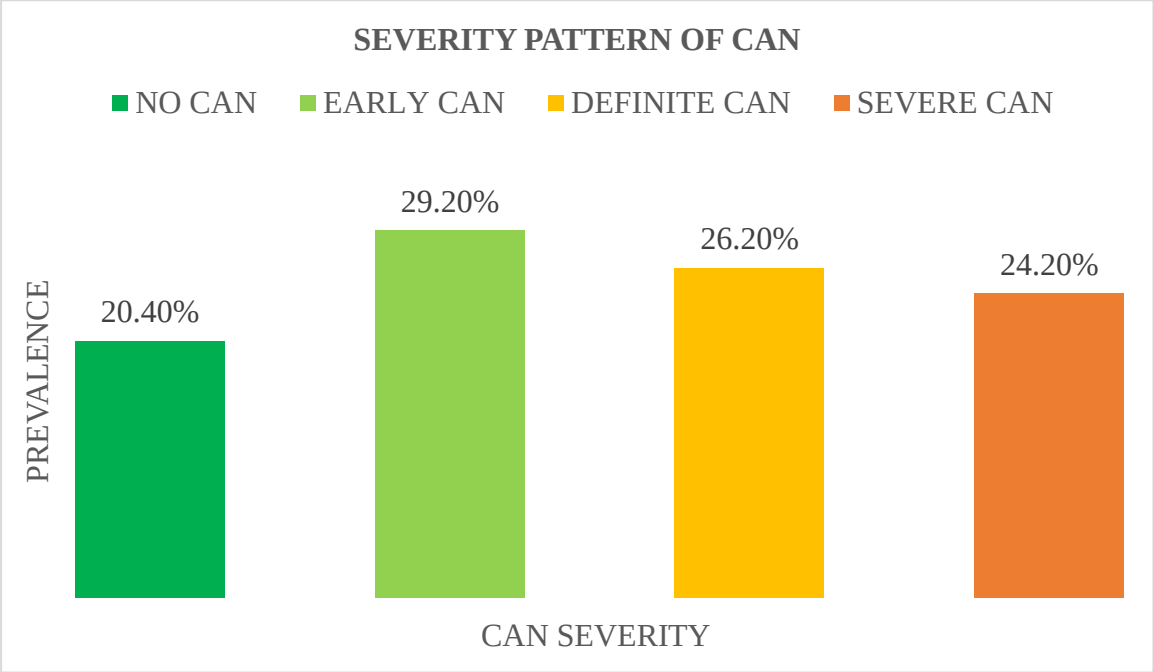

Figure 4. Graphical presentation of stratification of DCAN among T2DM at AURTH, Asella, Ethiopia, 2021, (n=260)

5.7 Factors associated with DCAN among T2DM patients

In binary logistic regression Age, sex, income level, residence, occupation, treatment type, regularly taking drugs, systolic blood pressure, and above, body mass index, fasting blood glucose, hypertension, nephropathy, retinopathy, Obstructive sleep apnea symptoms, history of alcohol consumption, and having the habit of smoking were associated with DCAN among DM type2 patients ($P < 0.25$).

After overall variables with a P value of less than 0.25 on bivariate analysis were entered into and analyzed by multivariate logistic regression at the same time only age above 60, longer duration of diabetes, poor glycemic control (raised FBG), hypertension, and retinopathy were significantly associated with DCAN($P < 0.05$).

Type2diabetic patients who were above 60 years old were 4.1 times more likely to have DCAN compared to those who were 60 years old or younger than 60. Type2diabetic patients with a duration of more than 10 years were 2.32 times more likely to develop DCAN when compared with those who were diabetic for 10 years or below l0 years [AOR (95 % CI) = 2.324(1.281-4.216)]. Type2diabetes mellites patients who had three-month average FBG > 126 mg/dl were 2.29 times more likely to develop DCAN compared to those whose three-month average FBG was70 - 126 mg/dl [AOR (95 % CI) = 2.287 (1.146 - 4.562)]. Type2 diabetes patients with hypertension were 1.98 times at risk of developing diabetic cardiac autonomic neuropathy when compared with those without hypertension. Type2 diabetes patients with retinopathy were around 2.02 times more likely to develop DCAN when compared with those without retinopathy. **(Table 5)**

Table 5. Bivariate and multivariable logistic analysis of factors associated with CAN among type2 diabetes mellitus patients AURTH, 2021, (n=260)

Variables	CAN status		Bivariate		Multivariate	
	-ve (%)	+ve N (%)	COR (95 % CI)	P value	AOR (95 % CI	P value
Age (year)						
> 60	20(22.7%)	68(77.3%)	5.883(3.270-10.582)	<0.001*	**4.106(2.182-7.725)**	**< .001****
< 60	10963.4%)	63(36.6%)	1		**1**	
Sex						
Male	74(44.8%)	91(55.2%)	1.691(1.015-2.816)	0.043*	.066(.544-2.089)	0.8531
Female	55(57.9%)	40(42.1%)	1		1	
Residence						
Urban	70(44.0%)	89(56.0%)	1.786(1.078-2.958)	0.024*	.486(.828-2.669)	0.1841
Rural	59(58.4%)	42(41.6%)			1	
Occupation						
Civil servant	102(47.7%)	112(52.3%)	1.560(.818-2.975)	0.177	1.491(.689-3.230)	0.311
others	27(58.7%)	19(41.3%)	1		1	
Income level						
<2000	19(39.6%)	29(60.4%)	1.646(.870-3.116)	0.126	1.591(.746-3.394)	0.229
>2000	110(51.9%)	102(48.1%)	1		1	
Type of treatment						
Insulin only	44(42.7%)	59(57.3%)	1.872(1.106-3.168)	.019*	1.547(.819-2.924)	0.179
Both	11(36.7%)	19(63.3%)	2.412(1.060-5.487)	.036*	1.222(.437-3.415)	0.702
OHA only	74(58.3%)	53(41.7%)	1		1	
Regularly take anti-diabetic medications						
NO	42(41.2%0	60(58.8%)	1.751(1.058-2.897)	0.029*	1.519(.851-2.712)	0.157
Yes	87(55.1%)	71(44.9%)	1		1	
Duration with diabetes						
>10 years	30(34.1%)	58(65.9%)	2.622(1.536-4.475)	<.001*	**2.324(1.281-4.216)**	**0.006****
≤10 years	99(57.6%)	73(42.4%)	1		**1**	
Systolic blood pressure in mmHg						
>139	29(34.9%)	54(65.1%)	2.418(1.409-4.151)	.001	1.235(.626-2.436)	0.543
80-139	100(56.5%)	77(43.5%)	1		1	
BMI						
>/=25	32(36.8%)	55(63.2%)	2.194(1.292-3.724)	0.004*	1.463(.767-2.791)	0.248
<25	97(56.1%)	76(43.9%)	1		1	
FBG:						
>126 mg/dl	87(43.7%)	112(56.3%)	2.846(1.546-5.237)	0.001*	**2.287(1.146-4.562)**	**0.019****
79-126 mg/dl	42(68.9%)	19(31.1%)	1		**1**	
Hypertension status						
Yes	38(33.9%)	74(66.1%)	3.109(1.862-5.190)	<0.001*	**1.980(1.108-3.540)**	**0.021****
No	91(61.5%)	57(38.5%)	1		**1**	
Nephropathy status						
Yes	33(36.3%)	58(63.7%)	2.311(1.368-3.906)	.002*	1.414(.739-2.703)	0.295
No	96(56.8%)	73(43.2%)	1		1	
Retinopathy status						
Yes	48(36.9%)	82(63.1%)	2.718(1.491-4.955)	0.001*	**2.024(1.152-3.556)**	**0.014****
No	81(62.3%)	49(37.7	1		**1**	
Obstructive sleep apnea symptoms						
Yes	44(40.7%)	64(59.3%)	1.845(1.119-3.042)	0.016*	1.083(.527-2.223)	0.829
No	85(55.9%)	67(44.1%)	1		1	
Alcohol consumption						
Yes	25(38.5%)	40(61.5%)	1.829(1.031-3.245)	.039*	1.610(.825-3.142)	0.163
No	104(53.3%)	91(46.7%)	1		1	
Smoking status						
Yes	12(38.7%)	19(61.3%)	1.654(.768-3.564)	.199	1.427(.528-3.857)	0.483
No	117(51.1%)	112(48.9%)	1		1	

Note: *Statistically significant, ** independent predictors, COR- Crude odds ratio; AOR- Adjusted odds ratio; CI- Confidence interval

Chapter Six: Discussion

Cardiovascular diseases are the major cause of morbidity and death in diabetics (DM), with cardiac autonomic neuropathy (DCAN) being one of the most prevalent but unappreciated complications of diabetes, even though it can be lethal (35). CAN caused by diabetes damages autonomic nerve fibers that innervate the heart and blood vessels, causing abnormal heart rhythm and vascular dynamics (51).

This is the first study in Ethiopia to use cardiac autonomic reflex tests (CARTs) or Ewing tests as a diagnostic tool to investigate the prevalence and risk factors of CAN among type 2 diabetic patients in Ethiopia at Arsi University Referral and Teaching Hospital. Physical examination, interviews, weight and height measurement, and Ewing tests all had 100% response rates. Religion, ethnicity, income, residence, occupation, marital status, type of medication, adherence to medication, nephropathy, systolic blood pressure, HIV infection, and substance use did not differ significantly between CAN negative and CAN positive type 2 diabetes patients. However, CAN was predicted by older age, longer duration of diabetes, poor glycemic control, retinopathy, and presence of hypertension among T2DM patients.

The prevalence of CAN was higher in this study (50.4 percent), which was substantially identical to the findings of studies conducted in India (53.2 percent) (93) and (52.82%) (74) with similar instruments (CARTs)or (Ewing tests). This is also in line with the Cameroonian systemic review that showed the number of patients with CAN varies from 27.5% to 73% in patients with DM type 2 (94). The higher prevalence of CAN among T2DM patients could be due to a higher number of people who were older and diabetic for a longer time, a higher prevalence of microvascular complications, and poor glycemic control, which leads to a lack of insulin or signaling disturbance in the nervous system, and/or the effect of hyperglycemia on autonomic nerve fibers that innervate the heart and blood vessels. Our CAN prevalence was significantly lower than that found in studies conducted in India, (68%)(94), and China 62.6%(93). Disparities in diagnosing methods, population geographical differences, and sample size could all be factors in this disparity. Our study, on the other hand, found a higher prevalence of CAN than previous Ugandan (22.7) (93) and Nigeria (29%) (12). This could be related to a population disparity; in our study, a substantial proportion of patients over 65 were included, whereas they (Ugandans) only allow individuals below 65 to participate.

The result of the present study showed that the prevalence of possible CAN was 29.2%; 26.2 percent of the patients had moderate (definite CAN and 24.2 percent of the patients had severe(advanced) CAN. Only 20.4% of the patients had no abnormality in cardiac autonomic function tests and so were categorized as normal. This is supported by an Indian study, that showed that the prevalence of early CAN account for 25%, definite CAN for 24%, and severe CAN account for 21% (12). This is higher than the result of a study conducted in Pakistan, in which the prevalence of CAN was determined to be 40%, with early, definite, and severe involvement identified in 13.9%, 12.5%, and 13.9 percent, respectively (16). According to the Indian study severity of CAN was found to be early in 20%, definitive in 45%, and advanced in 35% (18) and this is higher than our results.

In this study older age showed a statistically significant association with cardiac autonomic neuropathy. T2DM patients older than 60 were around 4.1 times more likely to have CAN than those who were 60 or younger. This is in line with a study conducted in Germany (50), China (12), Denmark (19), India (51), and Uganda (54). This may be due to the negative effects of aging on autonomic nerve fibers.

The incidence of cardiovascular illnesses rises with age, and this rise is attributable in part to the structural and functional dysregulation of the autonomic nervous system (ANS). Increased sympathetic output results in a long-term increase in circulating catecholamine levels, which is followed by cardiac βAR expression desensitization/dysfunction and downregulation. Another prominent feature of this scenario is the decline in the expression/activity of the norepinephrine transporter (NET) in sympathetic nerves that is liable for impaired reuptake of norepinephrine from the neuroeffector junction thus contributing to catecholamine spillover. Both the density and function of the M2 receptor decline with age, leading to a decrease in cardiac parasympathetic activity and thus accounting for diminished baroreflex activity with age. Marked reduction in autonomic nerve fiber density of both sympathetic and cholinergic compartments. Modulating cardiac function, and this deterioration is, at least in part, ascribable to a reduction in circulating BDNF levels (35).

The prevalence of CAN and diabetes duration had a significant association according to our findings. T2DM patients with a diabetic duration of > 10 years were 2.32 times more likely to develop DCAN compared with those with a diabetic duration of less than or equal to 10 years

which is in agreement with findings from studies done in Jordan (58), Ukraine (52), Saudi Arabia (7), Denmark (53) and south India(65).

Our study also showed that there is a significant relationship between CAN and poor glycemic control among type2diabetes. T2DM patients who had three-month average FBG >126 mg/dl were around 2.287 times more likely to have CAN than those who had a three-month average FBG $\leq$130. This is consistent with studies of Pakistan (54), Taiwan(77), Switzerland(52), America (35), Denmark(63), and Italy (12).

Poor glycaemic control and the duration of the DM diagnosis play an important role, both for triggering the physio-pathological mechanisms of CAN development (cellular destruction secondary to oxidative stress, accumulation of advanced glycosylation products, activation of the polyol dependent metabolic pathway, depletion of the nitric oxide in the microcirculatory endothelium that impairs the nerve vascularisations) and in the progression of the disease (27).

This study also revealed that there is a statistically significant association between the prevalence of CAN and hypertension among type2 diabetic patients. Type2 DM patients who had hypertension were 1.98 times more likely to develop CAN when compared with those who were none hypertensive. This is in agreement with the studies conducted in India(82), the UK(67), Saudi Arabia (33), Greece (72), and Egypt (46). The study conducted by the American Diabetes Association also showed that Intensive blood pressure (BP) intervention decreased CAN risk by 25(73).

Even though the nature of any relationship between DCAN and hypertension is far from clear hypertension has a synergistic effect with diabetes in CAN development (5). Essential hypertension acts synergistically with type 2 diabetes to depress cardiac reflex vagal and sympathetic function, and insulin resistance may play a pathogenic role in these processes(67). In HTN, owing to the fluctuations in BP, the baroreflex function gets hampered and is not able to set itself to a new operating point. Diminished sensitivity of baroreflex is a leading cause of autonomic imbalance in HTN. Although, some common pathways are related to the development of autonomic dysfunction in diabetes and HTN, CAN in diabetes is primarily hyperglycemia-mediated, and autonomic dysfunction in HTN is in part due to changes in the baroreflex mechanism.

Considering different etiologies behind autonomic dysfunction in diabetes and hypertension, it may be speculated that due to the co-existence of these different etiologies together in the

DMHTN group, further deterioration of autonomic function at rest and impaired autonomic recovery was found when compared to the normotensive diabetic group (62).

Our research also showed that CAN and retinopathy in T2DM patients have an independent connection (P= .014). T2DM patients with retinopathy were 2.02 times more likely than those without retinopathy to develop CAN, according to the logistic regression. This outcome is similarly consistent with previous studies of Brazil (95), Taiwan (91), Romania, and India(92). The pathophysiology is complex and poorly understood. Reduced capillary blood flow, basement membrane thickening, and the production of microaneurysms are considered to be caused by oxidative stress, inflammation, and the buildup of advanced glycation end products (82).

Limitation of the study

Because of the nature of the study cause and effect relationship between the variables was not established. Since the Valsalva maneuver was detrimental for patients with proliferative retinopathy and we didn't screen all patients for retinopathy because it is not feasible, we omitted this maneuver and replaced it with resting heart rate in standard Ewing test or cardiac autonomic reflex test.

CHAPTER SEVEN: CONCLUSION AND RECOMMENDATION

7.1 Conclusion

From the finding of this study, it is possible to conclude that CAN is common (50.04%) among type II diabetic patients and older age, presence of hypertension, longer duration of diabetes, poor glycemic control, and presence of retinopathy were significantly associated with DCAN among type II diabetes patients.

7.2 Recommendations

For Ministry of health

- ✓ To integrate screening strategies for CAN among DM patients as part of diagnostic modality.

For Clinicians

- ✓ Consider CAN screening in all diabetes patients during their regular follow-up especially in those with older age, longer duration of diabetes, poor glycemic control, retinopathy, and hypertension.

For T2DM patients

- ✓ To get screened for autonomic neuropathy as soon as possible to avoid or delay the catastrophic implications of the condition.

For researchers

- ✓ To undertake further study concerning the issue with relatively strong study designs like cohort and longitudinal.

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ANNEXES

Annex I: English version of the information sheet, consent form, and Questionnaire

Part One: English Version of the Information Sheet

Hello: Good morning /afternoon?

I would like to start by extending a sincere welcome. It is my pleasure to introduce myself, my name is _____I am a data collector of Mr. Abdulmalik Jeben Wako who is graduating student by Master of Science in medical physiology at the department of biomedical science institute of health, Jimma university. I am here today to collect data on *"Assessment of Cardiac Autonomic Neuropathy and Associated Factors Among Type 2 Diabetes Mellitus Patients at Arsi university Referral and Teaching Hospital, Southeast Ethiopia".*

Procedures

Your selection for this particular study is randomly and participation in this study is based on your voluntariness. You have a full right not to participate in this study; however, we encourage you to participate since your responses are very important to look at the magnitude and to identify factors associated with the development of diabetic cardiac autonomic neuropathy. If you agree to participate, you will be asked some general questions about your background, such as your age, marital status, education level, ethnicity, religion, and occupational status. You will also be asked specific questions on the risk factors and medications used. ECG will be done under the full Covid 19 protocol, which will help us to determine the cardiac autonomic reflex status. The interview and ECG procedure will last about 30 to 40 minutes.

Risks and discomforts

In this particular study, there are no procedures and questions that may harm or give you a feeling of discomfort. You can refuse to answer any question or stop the interview at any time. It is also your right not to give a response to some of our questions if you don't want to respond.

Benefits

What we will learn from the research will be used to recommend policymakers and health planners to appropriately design effective and accessible services to monitor the cardiac autonomic status of patients early. In the course of the interview, you may learn new information about diabetic cardiac autonomic neuropathy.

Confidentiality: We would like to assure you that privacy will strictly be maintained throughout. Your responses to any of the questions will not be given to anyone else and no reports of the study will ever identify you. You will be allowed to undress above the waist to allow access to the upper torso for accurate electrode placement in a private environment with minimal risk of interruption. The ECG will be correctly labeled with your identification number, relevant clinical details, and any variations to the normal recording conditions. ECG recordings that are digitally stored will be accompanied by a unique identifying number. All information on you will be treated privately. If a report of the results will be published, only Information about the total group will appear. Therefore, your honest and genuine responses are crucial for the success of this study. So, we kindly request you participate.

Procedures for handling adverse events: Appropriate measures to minimize the risk of infection transmission will be undertaken. Hands will be washed with soap and water or cleansed with alcohol gel before and after any contact with a patient. For patients requiring high levels of infection control precautions, personal protective equipment such as gowns, facemask, and gloves will be worn. Appropriate clinical waste disposal facilities will be available including sharps bins for the disposal of the single-use blade for a battery-operated razor or a single-use razor. All incidental findings identified during screening will be referred at the investigator's discretion to the appropriate additional care per the current standard of care.

Persons to contact: If you have any questions, you can contact the investigator at the following address and you may ask at any time you want.

Abdulmalik Jeben

Tel: 0921576044

Email address: Abdulmalik.jeben@gmail.com

May I have your permission to go to the consent form?

1. Yes..... (Continue)

2. No..... (Stop)

Part II: English Version of the consent form

According to the above information given to me regarding the objective of the study, procedures, risks, and discomforts, benefits of the study, and confidentiality of the responses, I agree to be interviewed for all the questions that the interviewer asks me and I approve with my signature. If the participant is unable to sign, please ask her/him to put inked thumbprints on the consent form.

Name and signature of the consenting interviewer_____, _____

Respondent signature_____

May I have your permission to proceed to the interview?

1. Yes..... (If yes, start the interview)

2. No..... (Thank you, stop here)

Result of the interview:

1. Completed

2. Partially completed

Supervisors name & signature_____, _____

The time that the interview has started_____

The time that the interview has completed_____

Part III: English Version of the Questionnaire

Participants ID _____

Instruction: circle the response from the alternatives

Table 6: Socio-demographic characteristics

Age (years)	_____	Religion	1. Orthodox
Sex	1. Male		2. Muslim
	2. Female		3. Protestant
Residence	1. Urban		4. Others (specify)____
	2. Rural	Ethnicity	1. Oromo
Educational status	1. No formal schooling		1. Amhara
	2. Primary completed		2. Gurage
	3. Secondary completed		3. Tigre
			4. Others (specify) _____
	4. College/university	Occupational status	1. Government employee
	5. Postgraduate degree		2. Self-employed
Marital status	1. Single		3. Housewife
	2. Married		4. Daily laborer
	3. Separated		5. Retired
	4. Divorced		6. Farmer
	5. Divorced		7. Other (specify)_____
		Level of income	1. High
			2. Medium
			3. Low

Table 7: Clinical status profile

Patient profiles	Responses	Patient profiles	Responses
Type of treatment	l. insulin	Duration with diabetes	1. <5 years
	2.OHA		2. 5-10 years
	3. Both		3. 10-15 years
Hypertension status	1. Yes		4. >15years
	2. No		
Nephropathy status	1. Yes	HIV/AIDS status	1. Reactive
	2. No		2. non-reactive
Retinopathy status	1. Yes		3. Unknown
	2. No	Weight of the patient (in kg)	_____
Obstructive sleep apnea symptoms	1. Yes		
	2. No	Height of the patient (in m)	_____
Systolic blood pressure	_____mmhg		
Diastolic blood pressure	_____ mmhg	BMI	_____kg/m^2
Regularly take Anti diabetic medications	1. Yes	FBG:	
	2. No	Resting heart rate	_____ppm

Table 8: Substance use assessment (alcohol intake, khat chewing, cigarette smoking)

Patient profile	Response
Alcohol consumption	1. Yes
	2. No
Smoking status	1. Smoker
	2. Non-smoker
Khat chewing status	1. Khat chewer
	2. non-chewer

Table 9: Symptoms of cardiac autonomic neuropathy

Symptoms	Responses	
Weakness	1.Yes	2.No
Dizziness	1. Yes	2.No
Frequent urination	1.Yes	2.No
Upper body perspiration	1. Yes	2.No
Nocturia	1.Yes	2.No
Obstructive sleep apnea	1.Yes	2.No
Palpitations	1.Yes	2.No
Syncope	1.Yes	2.No
Dyspnea	1.Yes	2.No
Chest pain	1. Yes	2.No
Awareness of the disease and its complications	1. Yes	2.No

Table 10: Diagnostic tests for cardiovascular autonomic neuropathy

Test	Normal (score 0)	Borderline (score 0.5)	Abnormal (score 1)
Resting heart rate	<100	100-110	>110
Heart rate response to deep breathing	≥15	11-14	≤10
The standing 30:15 ratio	≥ 1.04	1.01-1.03	≤ 1.00
Valsalva (V) Tests	≥1.21	1.11–1.20	≤1.10
Blood pressure response to standing: fall in systolic BP in mmHg	≤10	11-19	≥20
Blood pressure response to sustained handgrip: rise in diastolic BP in mmHg	>16	11- 15	≤10
CAN diagnosis	1.CAN^- <2 abnormal result		2.CAN^+ (≥2 abnormal results)
CAN severity	1. no CAN/normal	2. Early CAN	3. Definite CAN) 4. severe CAN)

Annex II: Amharic version of the information sheet, consent form and Questionnaire

ክፍል አንድ - የመረጃ ሉህ የአማርኛ ስሪት

ሰላም: ደህና ኣደሩ /ዋሉ?

ከልብ የመነጨ አቀባበል በማድረግ መጀመር እፈልጋለሁ። በራሴ ማስተዋወቅ ሲጅምር ደስታዬ ነው ፣ የእኔ ስም _____ እኔ በጅማ ዩኒቨርሲቲ ጤና ኢንስቲትዩት በባዮሜዲካል ሳይንስ ት/ት ክፍል በሕክምና ፊዚዮሎጂ በሳይንስ መምህር የሚመረቀው የአቶ አብዱልማሊክ ጀበን ዋቆ መረጃ ሰብሳቢ ነኝ ።

በጥናቱ ላይ ተጨማሪ ማብራሪያ መጠየቅ የሚፈልጉ ከሆነ የዚህ ጥናት ዋነኛ መርማሪ አቶ አብዱልማሊክ ጀቤን ማነጋገር ይችላሉ በሞባይል ስልክ ቁጥር 0921576044 በኩል, ወይም የኢሜይል አድራሻ Abdulmalik.jeben @ gmail.com።።

ክፍል II - የስምምነት ቅጽ የአማርኛ ስሪት

የጥናቱ ዓላማ ፣ የአሠራር ሂደቶች ፣ አደጋዎች እና ምቾት ፣ የጥናቱ ጥቅሞች እና የምላሾች ምስጢራዊነት በተመለከተ በተሰጠኝ መረጃ መሠረት ቃለ በጥናቱ ለመሳተፍ ተስማምቻለሁ።

የተሳታፍው ስም እና ፊርማ _____ ፣ _____

የመረጃ ሰብሳቢው ፊርማ _____

ክፍል III የመጠይቁ አማርኛ ስሪት

መመሪያ - ምላሾቹን ከአማራጮች ክበብ ያድርጉ

ሠንጠረዥ 3 -ማህበራዊ-ስነ-ሕዝብ ባህሪዎች

እድሜ	_____	ሀይማኖት	1. ኦርቶዶክስ
ጾታ	1. ወንድ		2. ሙስሊም
	2. ሴት		3. ፕሮቴስታንት
መኖሪያ	1. ከተማ		4. ሌላ (ጥቀስ)____
	2. ገጠር	ብሄር	8. ኦሮሞ
የትምህርት ሁኔታ	1. መደበኛ ትምህርት የለም		5. አማራ
	2. የመጀመሪያ ደረጃ ተጠናቀቀ		6. ጉራጌ
	3. ሁለተኛ ደረጃ ተጠናቀቀ		7. ትግሬ
			8. ሌላ (ጥቀስ) _____
	4. ኮሌጅ/ዩኒቨርሲቲ	የሙያ ሁኔታ	1. የመንግስት ሰራተኛ
	5. የድህረ ምረቃ ዲግሪ		9. በግልተዳዳሪ
የጋብቻ ሁኔታ	1. ያላገባ		10. የቤት እመቤት
	2. ባለትዳር		11. ቀን ስራተኛ
	3. የተለያዩ		12. ገበሬ
	4. የትፋቱ		13. ጡረተኛ
	5. የሞተበት		14. ሌላ (ጥቀስ)_____
		የገቢ ምጠን	1. ከፍተኛ
			2. መካከለኛ
			3. ዝቅተኛ

ሠንጠረዥ 4 የክሊኒካዊ ሁኔታ መገለጫ

የታካሚ መገለጫዎች	ምላሾች	የታካሚ መገለጫዎች	ምላሾች
የሕክምና ዓይነት	l. እንሱሊን	ከስኳር በሽታ ጋር የቆዩት ጊዜ	1. <5 ዓመታት
	2.ክንን		2. 5-10 ዓመታት
	3. ኡለቱም		3. 10-15 ዓመታት
የከፍተኛ ደእምግፊት ሁኔታ	1. ኣዎ		3.> 15 ዓመታት
	2. ኣይደለም		
የኩላሊት ችግች	1. ኣዎ	የኤች ኣይ ቪ/ኤድስ ሁኔታ	1. የተያዘ
	2. ኣይደለም		2. ያልተያዘ
የ አይን ችግች	1. ኣዎ		3. ያልታወቀ
	2. ኣይደለም	የበሽተኛው ኪብደት ብክሎግራም	_____
በእንቅልፍ ግዜ ምታፈን?	1. ኣዎ		
	2. ኣይደለም	የብሽተኛው ቁመት ብሜትር	
የላኛው ደም ግፊት	_____mmhg		_____
የታችኛው ደም ግፊት	_____ mmhg	BMI	_____kg/m^2
በመደበኛነት የፀረ -የስኳር በሽታ መድኃኒቶችን መውሰድ	2. 1. ኣዎ	FBG:	
	2. ኣይደለም	እረፍት የልብ ምት	_____ppm

ሠንጠረዥ 5 - የአደንዛዥ ዕፅ አጠቃቀም ግምገማ (አልኮሆል መጠጣት ፣ ጫት መቃም ፣ ሲጋራ ማጨስ)

የታካሚ መገለጫ	ምላሽ
የአልኮል መጠጥ	1. ኣዎ
	2. ኣይደለም
የማጨስ ሁኔታ	1. አጫሽ
	2. የማያጨስ
ጫት የመቃም ሁኔታ	1. ጫት የምቅም
	2. የማይቅም

ሠንጠረዥ 6 - የልብ ራስ -ሰር የነርቭ ህመም ምልክቶች

ምልክቶች	ምላሾች	
ድክመት	1. ኣዎ	2. ኣይ
መፍዘዝ	1. ኣዎ	2. ኣይ
ተደጋጋሚ ሽንት	1. ኣዎ	2. ኣይ
የላይኛው አካል ላብ	1. ኣዎ	2. ኣይ
የለልት ሽንት	1. ኣዎ	2. ኣይ
በእንቅልፍ ግዜ መታፈን	1. ኣዎ	2. ኣይ
ከፍተኛ ልብምት (ለራስ የምታወቅ)	1. ኣዎ	2. ኣይ
ራስን መሳት	1. ኣዎ	2. ኣይ
የመተንፈስ ችግር	1. ኣዎ	2. ኣይ
የደረት ህመም	1. ኣዎ	2. ኣይ
የበሽታውን እና ውስብስቦቹን ማወቅ	1. ኣዎ	2. ኣይ

ሥንጠረዥ 7 - የልብና የደም ሥር (የደም ቧንቧ) ራስ -ሰር የነርቭ በሽታ ምርመራ ምርመራዎች

ምርመራ	መደበኛ (ውጤት 0)	የድንበር መስመር (ነጥብ 0.5)	ያልተለመደ (ውጤት 1)
እረፍት የልብ ምት	70-100		> 100
በጥልቅ መተንፈስ የልብ ምት ምላሽ	≥ 15	11-14	≤ 10
የቆመው 30:15 ጥምርታ	≥ 1.04	1.01-1.03	≤ 1.00
ለመቆም የደም ግፊት ምላሽ -በ mmHg ውስጥ በሲስቶሊክ ቢፒ ውስጥ ይወድቃሉ	≤ 10	11-19	≥ 20
ለዘላቂ የእጅ መያዣ የደም ግፊት ምላሽ -በዲኤችኤች ውስጥ በዲያስቶሊክ ቢፒ ውስጥ መነሳት	> 16	11-15	≤ 10
የኢዊንግ ውጤት	<2 (CAN የለም)	2 (CAN)	> 2 (ከባድ CAN)

Annex III: Afan Oromo version of the information sheet, consent form, and questionnaire

Kutaa 1ffaa: oddeffannoo waliigalaa

Kaadhimamaa/tuu keenya ati akka gaafii fi deebii waa’ee midhamuu sirna narvii moggaa kan onnee kan dhukkuba sukkaraatiin walqabate dhufu irratti waliin taasisuuf carraan isin qaqabeera,irratti hirmaachuuf eeyyamamoodhaa?

Ashamaa harka fuune ,maqaan koo ----------jedhama.Ani waa’ee qo’annoo mata dureen isaa hariiroo dhukkuba sukkaaraafi midhamuu sirna nervii moggaa kan onnee gidduu jiru qo’atamuu irratti gaafii fi deebii isin wojjiin taasisuuf. Yeroo kamiiyyuu gaafii fideebii addaan kutuu mirga guutuu qabdu.

Lakk. Kaardii----------

Koodii hirmaatichaa----------iddoo hirmaataa----------

lakk. Bilbilaa----------mallattoo----------

Kutaa 2ffaa Gaafilee (questionaries Afan Oromo version)

Umrii	_____	Amantii	1. Oryodoksii
saala	1. Dhiira		2. Muslims
	2. Dhalaa		3. Protestaantii
Eddo jireenyaa	1. Magaalaa		4. Kan biraa (tarreessi)____
	2. Baadiyyaa	Ethnicity	15. Oromoo
Sadarkaa barnootaa	1. Hin baranne		9. Amaara
	2. sadarkaa 1ffaa		10. Guraagee
	3. Sadarkaa 2faa		11. Tigree
			12. Kan biraa (tarreessi)_____
	4. Kollejjii/Yunivarsiitii	Haala hojii	1. hojii mootummaa
	5. Digrii 2faafi isaa ol		16. Hojii dhuunfaa
Haala fudhaa fi heerumaa	1. kan hin fuudhin/herumin		17. Haadha manaa
	2. kan fuudhe/heeumte		18. Hojjetaa Hmnaa
	3. Kan gargar ba'an		19. Soorama
	4. kan wal kiikan		20. Qonnaan bulaa
	5. kan irraa du'e/duute		21. Kan biraa (tarreessi)_____
		Haala galii	1. Ol'aanaa
			2. Jidddu galeessa
			3. Gad'aanaa

Haala dhukkubsataa	Deebii	Haala dhukkubsataa	Deebii
Qoricha	l. insuliinii	Turtii dhukkuba sukkaaraa	1.waggaa 5 gadi
	2.OHA(kiniina)		2. wagga 5-10
	3. lamaanuu		3. wagga 10-15
Dhiibbaa dhiigaa ol'aanaa	1. Eeyyee		3. >wagga 15 oli
	2. Miti		
Dhibee kalee	1. Eeyyee	HIV/AIDS	1. pozitiivii
	2. Miti		2. nagatiivii
Dhibee ijaa	1. Eeyyee		3. hin beekamne
	2. Miti	Ulfaatina qaamaa (kg)	
Yeroo hirribaa ukkaamamuu	1. Eeyyee		_____
	2. Miti	Dheerina qaamaa (m)	
Dhiibb dhiigaa kan gubbaa	_____mmhg		_____
Dhiibbaa dhiigaa kan jalaa	_____ mmhg	Alkkolli dhugduu	1. Eeyyee
			2. Miti
Dawaa yeroon eeganii fudhachuu	1. Eeyyee	Tamboo xuuxuu	1. Eeyyee
			2. Miti
	2. Miti	Jimaa qaamuu	1. Eeyyee
			2. Miti

Mallattoolee	Deebii	
Dahhabbii	1.Eeyyee	2. Miti
Jaanja'uu	1.Eeyyee	2. Miti
Ammaa amma fincaa'uu	1.Eeyyee	2. Miti
Qaamni gara olii fuuruu	1.Eeyyee	2. Miti
Fincaan halkanii	1.Eeyyee	2. Miti
Yeroo hirribaa ukkaamamuu	1.Eeyyee	2. Miti
Dhahannaan onnee ofii namatti dhagahamuu	1.Eeyyee	2. Miti
Of wallaaluu	1.Eeyyee	2. Miti
Hafuura baafachuu dadhabuu	1.Eeyyee	2. Miti
Dhukkubbii laphee	1.Eeyyee	2. Miti
Dhukkubichaa fi rakkoolee isaa hubachuu	1.Eeyyee	2. Miti

Annex IV: Declaration

ASSURANCE OF PRINCIPAL INVESTIGATOR

I, the under signed, MSc student declare that this thesis is my original work in partial fulfillment of the requirements for the Master of Medical Physiology in which this has never been presented in this or any other University and that all sources of materials used for the thesis have been fully acknowledged.

Name of the student: Abdulmalik Jeben Wako

Date. _____ Signature _____

Approval of the first advisor

Name of the first advisor: Mr. Teshome Gobena

Signature: _____Date: _____

Approval of the second advisor

Name of the second advisor: Mr. Moyeta Beriso

Signature: _____Date: _____

Approval of the internal examiner

Name of the internal examiner: Mr. Elias Mulat (MSc, Assistant professor of physiology)

Signature: _____ Date: _____