ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4

Journal of Coastal Life Medicine

A Study on a Novel Biomarker in People with Type 2 Diabetes Who Have Cardiac Autonomic Neuropathy

Received: 24 October 2022, **Revised:** 23 November 2022, **Accepted:** 26 December 2022

Dr. K.M. Vaghasiya.
Tutor. Krishna Institute of Medical Sciences, Krishna Vishwa Vidyapeeth "Deemed to Be University",Karad – 415110,Maharashtra

Dr. D.V. Sarvaiya.
Tutor. Krishna Institute of Medical Sciences, Krishna Vishwa Vidyapeeth "Deemed to Be University", Karad –415110, Maharashtra

Dr.P.S Salve.
Assistant Professor Krishna Institute of Medical Sciences, Krishna Vishwa Vidyapeeth "Deemed To Be University",Karad – 415110,Maharashtra

Key Words:
Cardiac Autonomic Neuropathy, Novel Biomarker, Type 2 Diabetes

Abstract:
The purpose of this study is to examine the new biomarker in individuals with type 2 diabetes and cardiac autonomic neuropathy. The research was cross-sectional in nature. From May 2020 to November 2021, 211 patients were involved in this study. The current study's findings corroborate previous research showing that serum NSE levels are dramatically increased in T2DM with CAN. A biomarker with promise and a possible role in the diagnosis of CAN patients is elevated blood NSE levels.

1. Introduction:

India has the highest number of persons with diabetes worldwide, at 41 million. Cardiac autonomic neuropathy (CAN) is an often-ignored yet potentially fatal complication of diabetes mellitus (DM). The reported annual incidence of CAN in people with type 2 diabetes is 2%, and its prevalence varies from 31% to 73%. Multiple factors contribute to the complex history of CAN's origin. The symptoms of CAN don't appear until the latter stages of the disease, and the early stages are asymptomatic. Early detection of patients with May allows for the initiation of intensive treatments aimed at enhancing the quality of life, glucose control, and cardiovascular risk factors of those affected. There has been almost no research done on CAN among Indians, and none at all in the Northeast. Therefore, this study aims to investigate how often CANs play a role in T2DM and what factors contribute to this elevated risk. [1]

Cardioautonomic neuropathy (CAN) is a microvascular complication of diabetes that causes a seemingly innocuous impairment of cardiovascular autonomic function. It is estimated that 2% of persons with newly diagnosed or well-controlled diabetes have CAN, whereas as many as 60% of those with long-standing type 2 diabetes mellitus and 90% of those with type 1 diabetes who are candidates for a pancreas transplant have CAN. This wide variation in prevalence may be attributed to the wide variety of evaluation methods used to classify CAN, making it challenging to compare epidemiological data from different studies. CAN is more common in older people, those who have had diabetes for a long period, and those who have poor glycemic control [2, 3].

Silent myocardial ischemia, chronic renal illness, myocardial dysfunction, severe cardiovascular events, cardiac arrhythmias, and sudden death are all risk factors for CAN years before any symptoms appear [4]. It's also associated with a higher risk of diabetes-related complications and mortality. Obstructive sleep apnea (OSA), hyperglycemia, insulin resistance, prediabetes, obesity, high blood pressure, abnormal lipid profiles, metabolic syndrome, and other

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

conditions all have a role in the complicated pathophysiology of CAN [5]. While both type 1 and type 2 diabetes mellitus have substantial complications, diabetic neuropathy is the most prevalent chronic microvascular consequence of diabetes. Autonomic neuropathies (primarily CAN) and distal symmetric polyneuropathy [6] have been the primary focus of study.

It stands to reason that the incidence of should would increase as the global diabetes epidemic progresses. As of this year (2019), there are 463 million people with diabetes mellitus in the world. By 2045, the global prevalence of diabetes is expected to reach 10.9%, with 700 million people afflicted, according to the International Diabetes Federation. Given that diabetes was responsible for the deaths of 1.6 million people in 2021, placing it sixth on the list of leading causes of death worldwide, this is cause for alarm. 90% of people with type 2 diabetes are also overweight or obese, so it's crucial to keep that in mind. As a consequence of the burden of these chronic illnesses, the incidence of diabetes mellitus, obesity, and metabolic syndrome has surged, creating a cardiometabolic catastrophe. Therefore, it may be vital to reduce the number of fatalities due by this chronic pandemic to diagnose CAN early and start treatment. The goal of this study was to increase knowledge of CAN among healthcare providers by evaluating the most recent data on the condition's epidemiology, pathophysiology, and clinical assessment. [7]

CAN in diabetes

May is a severe condition that usually affects people who have had diabetes for a long period, however it may arise in those who haven't yet been diagnosed with diabetes. The majority of patients present with resting tachycardia, orthostatic hypotension, dizziness, loss of vision, syncope, and an inability to tolerate physical exertion [7]. Persistent tachycardia in diabetics has been related to vagus nerve damage. Bradbury and Eggleston first described orthostatic hypotension and tachycardia as a clinical disease in 1925, and by 1945, Rundles had confirmed comparable physiological abnormalities as manifestations of diabetic neuropathy. Research conducted on the probable late-stage effect of CAN, cardiac autonomic denervation, has shown an association with increased mortality [8, 9].

Total cardiac denervation, the loss of sympathetic and parasympathetic innervation that results in a decreased heart rate response, is a rare complication of diabetic neuropathy. Vagal denervation is more common and occurs earlier in development than sympathetic denervation [10]. As a result, the sympathetic and parasympathetic branches of the autonomic nervous system, which regulate heart rate variability and vascular dynamics, are affected. Sympathetic and parasympathetic autonomic nervous system (SANS) interaction produces sympathovagal balance, which controls the sinus node, promotes heart rate adjustments, modulates chronotropism, dromotropism, bathmotropism, and inotropism, alters systolic and diastolic volumes, and promotes control of vascular smooth muscle cells, which contributes to peripheral vascular resistance [11].

Autonomic dysfunction occurs when there are persistent disturbances to the equilibrium between the SANS and PANS. The intrinsic cardiac innervation and the rest of the autonomic nervous system are vulnerable to damage caused by degenerative, inflammatory, ischemic, and metabolic illnesses, which contribute to autonomic dysfunction. Autonomic cardiovascular dysfunction has the potential to raise the risk of atrial and ventricular arrhythmias and sudden cardiac death. The misconception that CAN is a subclinical condition increases the risk of mortality due to late identification [12], despite the fact that CAN development is now recognized as an independent prognostic factor for cardiovascular disease. Vital to lowering CAN-related morbidity and mortality is a firm grasp of the pathophysiological mechanisms that originate the disease, as well as the currently available and proposed clinical diagnostics [13].

Pathophysiology

CAN causes anomalies in cardiovascular autonomic regulation because to damage to the autonomic nerve fibers that normally supply the heart and blood vessels. This harm is brought about by an intricate web of interrelated pathophysiological processes. Hyperglycemia is the underlying cause of CAN, albeit the specific mix of risk factors for its development is yet unknown. The generation of ROS and AGEs, two

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

byproducts of the glycation process, is increased in people with hyperglycemia [14].

The presence or absence of glucose in food directly influences the non-enzymatic Maillard reaction, which produces AGEs from the carbonyl groups of reducing sugars and the free amino groups of proteins. This process may be reversed in its early stages but not later on. After being created, AGEs accumulate in the cellular environment. Advanced glycation end products (AGEs) have been linked to the activation of nuclear factor kappa B (NF-B) via the inhibition of phosphatidylinositol-3 kinase (PI3-K) and the activation of mitogen-activated protein kinases (MAPK).

Cardiomyocytes, neurons, adipocytes, vascular cells, immunological cells, glomerular epithelial cells, and lung epithelial cells all contain NF-B, which promotes membrane RAGE activation [15]. By stimulating the production of inflammatory cytokines including tumor necrosis factor and interleukin-6, this transcription factor encourages leukocyte transendothelial migration. 6. Hyperglycemia from type 1 and type 2 diabetes isn't the only thing that may enhance NF-B and pro-inflammatory cytokine production. NF-B contributes to the obesity-related inflammation and insulin resistance [16, 17].

Another factor that might influence CAN's development is the availability of operating systems. Breathing stops repeatedly throughout the night are a hallmark of obstructive sleep apnea (OSA), a sleep disease. In many cases, this is accompanied by a roaring snore. It is unclear how or why this disease causes hypoxic events, which in turn cause increased oxidative stress and the growth of CAN. Furthermore, obstructive sleep apnea (OSA) is prevalent in the diabetic population and is linked to an increased risk of cardiovascular morbidity [18].

2. Material and Methods:

Study Design

A hospital in Jaipur served as the site for this study's cross-sectional component. From May 2020 to November 2021, 211 patients were involved in this study. Each patient was evaluated on the basis of their symptoms and results from the Ewing autonomic function test. There were two groups of T2DM patients: 62 patients with CAN (the CAN patients group) and 140 T2DM patients without CAN (9 individuals in the non-CAN group dropped out of the trial owing to financial concerns). Patients from both the hospital's outpatient clinic and the emergency room participated in the research. Subjects required to be at least 18 years old, of either sex, and diagnosed with type 2 diabetes to participate.

Clinical feature measurement

At the time of enrollment, participants' seated right arm blood pressure was taken using a sphygmomanometer. Before eating breakfast, we all stood on the same scales to get our weight and the same wall-mounted stadiometer to get our height. Individual body mass indexes were then determined using the formula: kg/m 2. After 10 minutes of resting, sphygmomanometer readings were taken from each subject's right arm. Plasma glucose, glycated haemoglobin (HbA1c), lipid profile, and another clinical indicator were measured from fasting blood. High performance liquid chromatography (HPLC) was used for quantitative quantification of HbA1c. Ophthalmologists used a variety of methods, including a clinical examination and visual acuity tests, to diagnose retinal disorders.

Neuropathy assessment

The skilled doctor and tech followed established protocols while conducting the neuropathy tests. The severity of neuropathy was determined by testing responses to a monofilament, a pinprick, an ankle reflex, and a vibration (VPT) test.

Autonomic function test

To verify the CAN diagnosis, a Ewings autonomic function test was performed. Three of the five tests of autonomic function, including the heart rate response to deep breathing (beat-to-beat fluctuation), standing (30:15 ratio), and the Valsalva procedure, focused on the parasympathetic function. Blood pressure (BP) response during a change in posture and a handgrip test were used to evaluate sympathetic function.

Sample collection

At the start of therapy at the study center, 5 ml of fasting blood was taken from each participant. These samples were taken in anticoagulant-free vials and stored upright for an hour. Next, serum was extracted

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

by centrifuging the samples at 4,000 rpm for 15 minutes. Pipettes were used to collect the supernatant serum, which was then frozen at -800C for further biochemical analysis.

Statistical Analysis

Both discrete and continuous variables were used in the analysis. The categorical variables were shown as frequencies with percentages, while the scale variables were shown as means and standard deviations. The Shapiro-Wilks test was used to check the normality of all continuous variables. The Chi-square test and the Student's t-test were used to compare the categorical and continuous variables, respectively. Time to onset of cardiac autonomic neuropathy was analyzed using Kaplan- Meier survival curves and compared using the log-rank test. The statistical significance level was set at the p0.05 two-sided critical area. SPSS (New York, USA) version 21.0 was used for all analyses.

3. Results:

Characteristics of participants

Test results for autonomic nervous system activity (Figure 1) classified 140 of 202 T2DM patients (69.30%) as having T2DM without CAN, 7 of 62 patients (11.29%) as having early CAN, 24 of 62 patients (38.50%) as having definite CAN, and 31 of 62 patients (50.0%) as having severe CAN. There were 62 new instances of CAN, for a total prevalence of 30.7%. There was a statistically significant difference between the non-CAN and CAN patients group in terms of blood pressure (BP) fluctuation during deep breathing (HRD), standing (HRS), posture change, and the hand grip test.

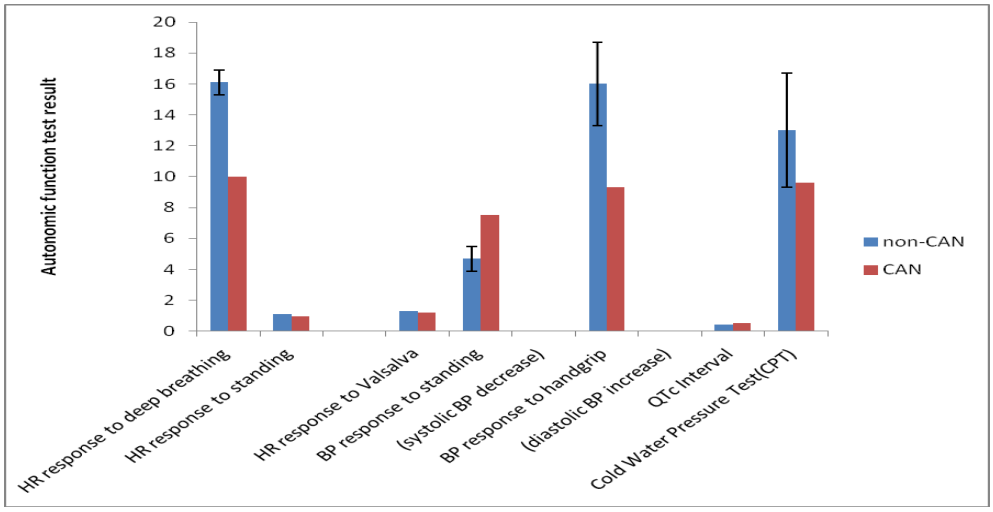

Figure 1: The findings of a cold water pressure test, a Qtc interval, and a test of autonomic function were also analyzed

Age Association, duration

Patients with CAN had a mean age of 55.7 ± 10.0 years, whereas those without CAN had a mean age of 51.3 ± 10.2 years. Additionally, it was discovered that 3.2% (2/62) of CAN patients were younger than 40 years old, 22.58% (14/62) of CAN patients were between the ages of 40 and 49 years old, 27.4% (17/62) of CAN patients were between the ages of 50 and 59 years old, 387.0% (24/62) of CAN patients were between the ages of 60 and 69 years old, and 8.06% (2/62) of CAN patients were older than 70 years old. The CAN group seemed to have diabetes for a longer period of time. Of the 62 CAN patients, 43.54 percent (27 of them) had T2DM for less than five years, 35.48 percent (22 of them) for six to ten years, 16.12 percent (10 of them) for 11 to fifteen years, and 4.8 percent (three of them) for more than fifteen years. The median age of diabetes diagnosis and the time it took for the CAN to be developed were compared. There were no discernible differences between the sexes. To back up the prior conclusion, a Kaplan-Meier analysis (Figure 2) was drawn.

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

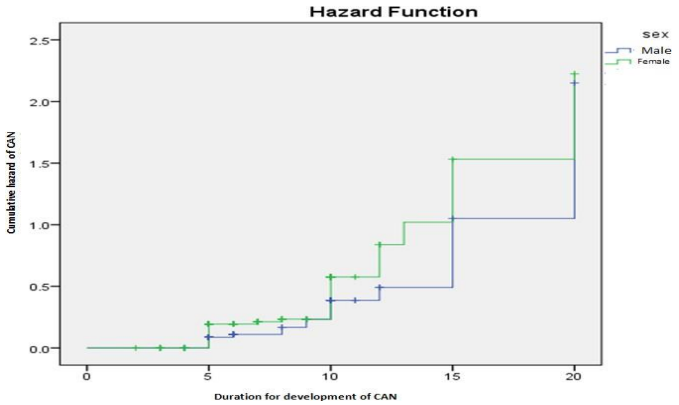

Figure 2: Kaplan–Meier curves from diagnosis of type 2 diabetes mellitus period to the establishment in men and women of Cardiac autonomic neuropathy

Participant's NSE levels

Differences in mean serum NSE levels between CAN and non-CAN were statistically significant (Figures 3, 4). Patients with CAN had a mean blood level of NSE of 11.48±1.31 ng/ml, whereas those without CAN had a level of 10.00±0.71 ng/ml. In this work, we found that NSE has the potential to serve as a new biomarker for CAN. Serum NSE levels were found to be higher in the CAN than in the non-CAN, with a mean value of 8.41± in the control group. Furthermore, there was no discernible correlation between the presence and lack of DPN and TyG index values.

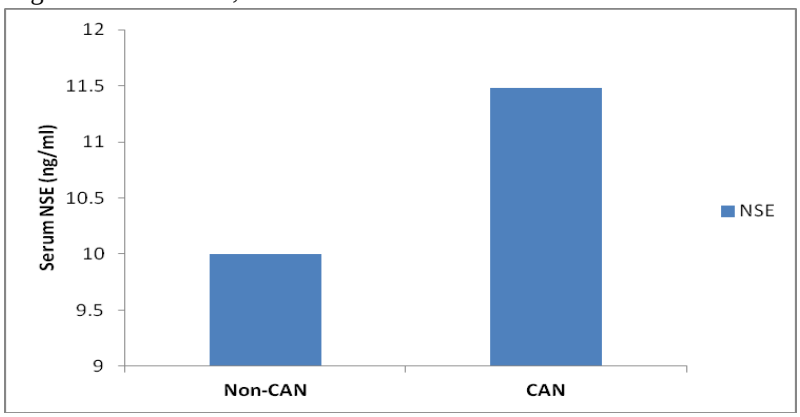

Figure 3: NSE level Graphical representation in Non-CAN and CAN

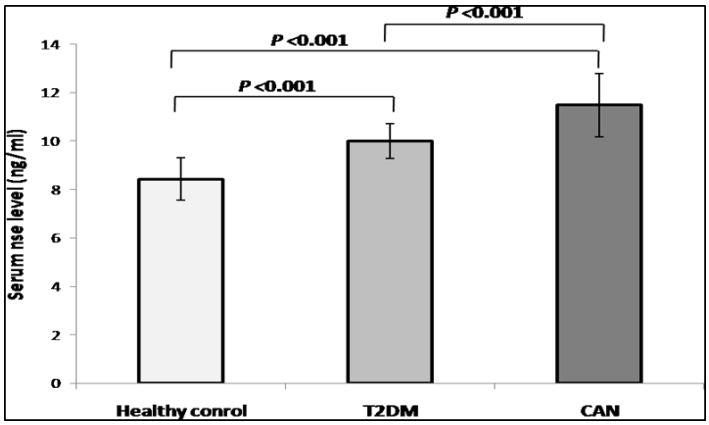

Figure 4: NSE level Graphical representation in Non-CAN and CAN when compared toHealthy Volunteer

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

NSE Index

There was a statistically significant difference between the NSE indices of the CAN and non-CAN groups, as well as the healthy control group. Also, women had a higher NSE index than males did typically (Figures 5 and 6), although the difference was not significant.

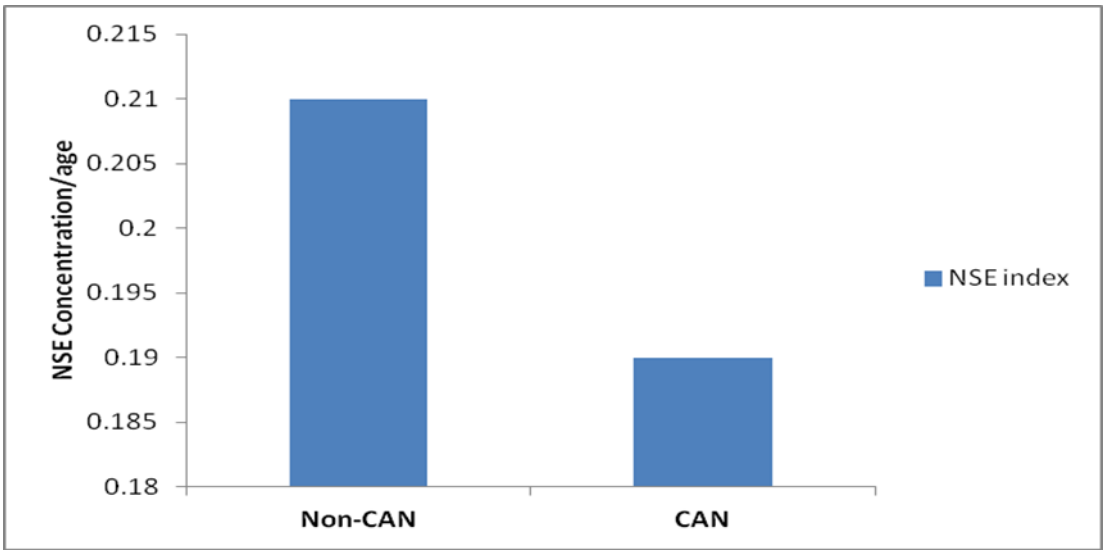

Figure 5: Non-CAN and CAN group NSE index

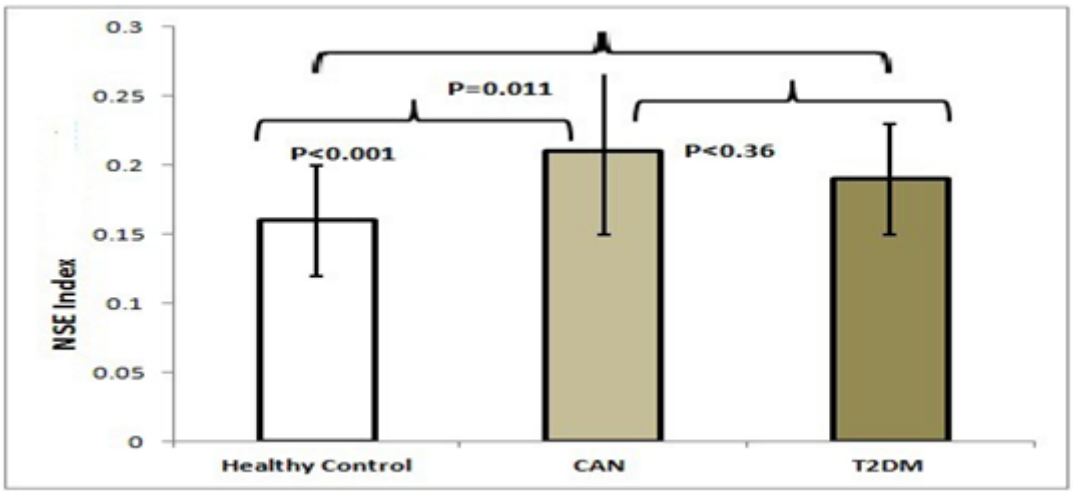

Figure 6: Non-CAN and CAN group NSE index compared to Healthy volunteer

Patient's TyG Index

Figure 7 displays the statistically significant (P <0.001) difference in TyG index between the CAN and non-CAN patient groups. When the TyG index for mild, moderate, and severe CAN was compared with that of the T2DM group, a statistically significant difference was found. Furthermore, there was no significant difference in TyG index levels between CAN and non-CAN individuals based on the presence or absence of DPN (TyG index in DPN it was 10.12 vs. 9.79, P = 0.06).

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

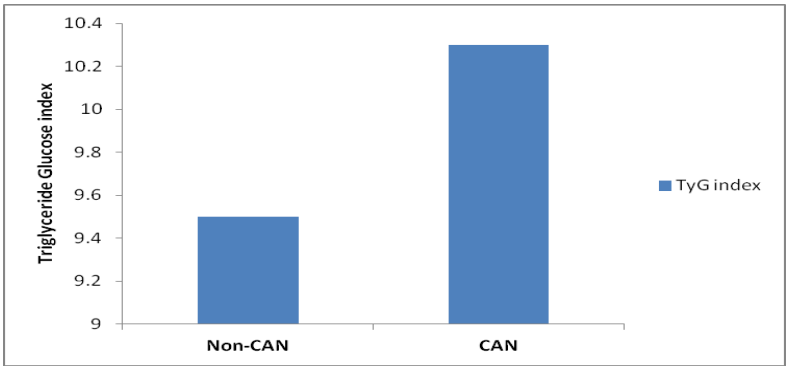

Figure 7: non-CAN and CAN group TyG index

Patient's GE Index

Figure 8 shows that there was a statistically significant (P<0.001) difference between the CAN and Non-CAN groups in terms of the GE index. The GE index of male patients was significantly higher than that of female patients.

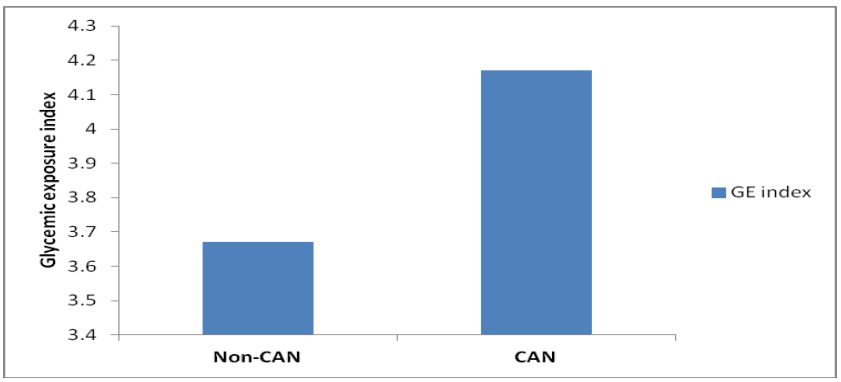

Figure 8: Non-CAN and CAN group GE index

TG/HDL ratio

As can be seen in Figure 9, there is a very significant (P <0.001) correlation between the TG/HDL ratios of the CAN and Non-CAN groups. When male and female patients' TG/HDL indices were evaluated independently, no statistically significant difference was found.

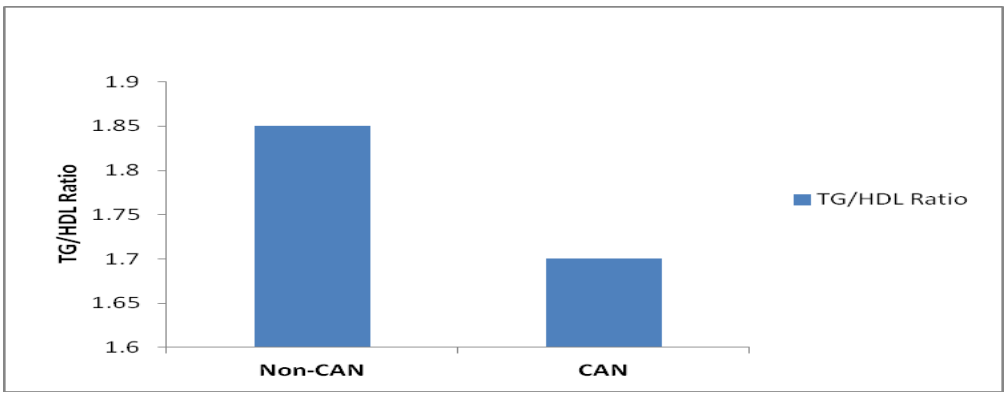

Figure 9: Non-CAN and CAN group TG/HDl ratio

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

Medication modalities

The use of insulin, oral hypoglycemic agents, and angiotensin-converting enzyme inhibitors/angiotensin receptor blockers was significantly lower in the CAN group compared to the T2DM group.

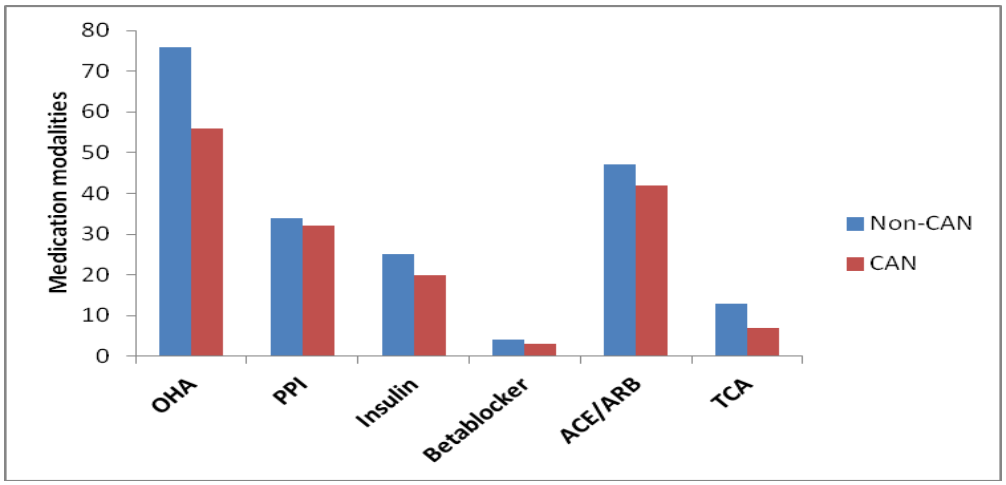

Figure 10: Treatment module of T2DM patients

4. Discussion:

Long-term inadequate glycemic management is now well recognized as having a significant influence on the onset and progression of CAN. One study found a link between high fasting triglycerides and diabetic neuropathy [19]. With the rising numbers of people suffering from obesity, metabolic syndrome, and type2 diabetes, hypertriglyceridemia has emerged as a serious health issue. Previous research has focused on insulin resistance and metabolic disease, such as CAN, but has employed a small sample size [20].

The current findings suggest that body mass index (BMI), alcohol use, and smoking are all contributors to the development of CAN. In individuals with type 2 diabetes, the recommendations of the Toronto consensus committee [21] about the correlation between BMI and CAN are supported by this study. In type 2 diabetes mellitus, macrovascular risk factors including as body mass index (BMI), pulse pressure (BP), and tobacco use have a larger role in the development of CAN.

The triglyceride glucose index (TyG index) was used as a low-cost substitute for HOMA-IR in the current investigation to assess insulin resistance. Since we looked into neuron-specific enolase in diabetic cardiac autonomic neuropathy, it adds credibility to the current work and piques interest because of its neurological basis. TyG index and neuron specific enolase investigation as a potential biomarker in diabetic CAN are two other unique entities discovered in the current study that point researchers in a new direction in their hunt for novel biomarkers in a wide range of diabetic complications, including cardiac autonomic neuropathy.

5. Conclusion:

The current study's findings corroborate previous research showing that serum NSE levels are dramatically increased in T2DM with CAN. An increased serum NSE level has diagnostic use and may serve as a biomarker for CAN. In this research, the overall prevalence of CAN was 30.7%. The significance of the TyG index assessment is also highlighted in the current investigation. Since this approach of measuring insulin resistance is very inexpensive, it has the potential to be used as a screening instrument for people at high risk of developing diabetic neuropathy. The presence of insulin resistance was also shown to be a separate risk factor for type 2 diabetes and its complications. TyG Index may be used in conjunction with the GE Index and the TG/HDL ratio to estimate insulin resistance.

References:

[1] Vinik AI, Maser RE, Mitchell BD, Freeman R. Diabetic autonomic neuropathy. Diabetes Care. 2003; *26*: 1553–1579.

[2] Maser RE, Mitchell BD, Vinik AI, Freeman R. The association between cardiovascular autonomic neuropathy and mortality in

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

individuals with diabetes: a meta-analysis. Diabetes Care. 2003; *6*: 1895–1901.

[3] Maser R, Lenhard M, DeCherney G. Cardiovascular autonomic neuropathy: the clinical significance of its determination. Endocrinologist. 2010; *10*: 27–33.

[4] Schumer MP, Joyner SA, Pfeifer MA. Cardiovascular autonomic neuropathy testing in patients with diabetes. Diabet Spectr. 2018; *11*: 227–223.

[5] Ziegler D, Zentai C, Perz S, Rathmann W, Haastert B, Meisinger C, Lowel H. Diminished heart rate variability (HRV) and prolonged QTc interval, but not increased QT dispersion (QTD) are predicators of mortality in the diabetic population. Diabetes. 2004; *53* (suppl 2): A57.

[6] Ziegler D, Zentai C, Perz S, Rathmann W, Haastert B, Meisinger C, Lowel H. Selective contribution of diabetes and other cardiovascular risk factors to cardiac autonomic dysfunction in the general population. Exp Clin Endocrinol Diabetes. 2006; *114*: 153–159.

[7] Witte DR, Tesfaye S, Chaturvedi N, Eaton SE, Kempler P, Fuller JH. Risk factors for cardiac autonomic neuropathy in type 1 diabetes mellitus. Diabetologia. 2020; *48*: 164–171.

[8] Ewing DJ, Clarke BF. Diabetic autonomic neuropathy: present insights and future prospects. Diabetes Care. 2018; *9*: 648–665.

[9] Vinik A, Erbas T, Pfeifer M, Feldman M, Feldman E, Stevens M, Russell J. Diabetic autonomic neuropathy. In: Porte D Jr, Sherwin RS, Baron A, eds. Ellenberg & Rifkin's Diabetes Mellitus. 6th ed. New York, NY: McGraw-Hill; 2013: 789–804.

[10] Kahn JK, Zola B, Juni JE, Vinik AI. Radionuclide assessment of left ventricular diastolic filling in diabetes mellitus with and without cardiac autonomic neuropathy. J Am Coll Cardiol. 2019; *7*: 1303–1309.

[11] Vinik A, Erbas T. Neuropathy. In: Ruderman N, Devlin JT, Schneider S, Kriska A, eds. Handbook of Exercise in Diabetes. Alexandria, Va: American Diabetes Association; 2012.

[12] American Diabetes Association. Standards of medical care in diabetes—2016. Diabetes Care. 2006; *9* (suppl 1): S4–S42.

[13] Colberg S, Swain D, Vinik A. Use of heart rate reserve and rating of perceived exertion to prescribe exercise intensity in diabetic autonomic neuropathy. Diabetes Care. 2013; *26*: 986–990.

[14] Position paper. Orthostatic hypotension, multiple systems atrophy (the Shy Drager Syndrome). J Auton Nerv Syst**.** 1996; *58*: 123–124.

[15] Albers AR, Krichavsky MZ, Balady GJ. Stress testing in patients with diabetes mellitus: diagnostic and prognostic value. Circulation. 2016; *113*: 583–592.

[16] Burgos LG, Ebert TJ, Asiddao C, Turner LA, Pattison CZ, Wang-Cheng R, Kampine JP. Increased intraoperative cardiovascular morbidity in diabetics with autonomic neuropathy. Anesthesiology. 2020; *70*: 591–597.

[17] Kitamura A, Hoshino T, Kon T, Ogawa R. Patients with diabetic neuropathy are at risk of a greater intraoperative reduction in core temperature. Anesthesiology. 2012; *92*: 1311–1318.

[18] Sobotka PA, Liss HP, Vinik AI. Impaired hypoxic ventilatory drive in diabetic patients with autonomic neuropathy. J Clin Endocrinol Meta*b.* 2014; *62*: 658–663.

[19] Wiggin, TD., Sullivan, KA., Pop-Busui, R., Amato, A., Sima, AA., Feldman, EL. Elevated triglycerides correlate with progression of diabetic neuropathy. Diabetes 2009; 58:1634-40.

[20] Lee, KE., Ji, Sun Nam., Chul, Woo Ahn., Ji-Man, Hong., Seung-Min, Kim ., Il-Nam ,Sunwoo.,Joon-Shik, Moon ., Sang-Jun, Na., Young-Chul, Choi. Insulin resistance is independently associated with peripheral and autonomic neuropathy in Korean type 2 diabetic patients. Acta Diabetol 2012; 49:97–103.

[21] Spallone, V., Ziegler, D., Freeman, R., et al. Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management. Diabetes Metab Res. 2011; 27:639-653.