"STUDY OF CARDIAC AUTONOMIC NEUROPATHY IN TYPE 2 DIABETES MELLITUS"

By

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IN

GENERAL MEDICINE

Under the guidance of

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Dr. A.N.L.N MURTHY

ABSTRACT

STUDY OF CARDIAC AUTONOMIC NEUROPATHY IN TYPE 2 DIABETES MELLITUS

Background:

Cardiac Autonomic Neuropathy (CAN) represents a significant cause of morbidity and mortality in diabetic patients and is associated with a high risk of cardiac arrhythmias and sudden death, possibly related to silent myocardial ischemia. Perhaps one of the most overlooked of all serious complications of diabetes is CAN. Cardiac autonomic function should be assessed in most diabetic patients as it contributes to the evaluation of cardiovascular risk. The presence of CAN should lead to further awareness of possible complications and optimal control of risk factors. Knowledge of early autonomic dysfunction can encourage patient and physician to improve metabolic control and to use therapies such as ACE inhibitors and beta blockers, proven to be effective for patients with CAN.

Objectives:

1. To assess the cardiac autonomic function in patients with Type2 Diabetes Mellitus (T2DM) for detection of CAN.
2. To evaluate the relationship between CAN and duration of diabetes, and glycemic control in Type 2 DM.

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Materials and Methods:

This is a case-control study conducted in Sri Devaraj Urs Medical College, Tamaka, Kolar. Sixty patients (Cases) of T2 DM aged > 40 years admitted/visiting Diabetic clinic in R.L Jalappa hospital and sixty healthy non diabetic controls matched for age and sex and satisfied the inclusion and exclusion criteria and gave informed consent for the study were included in the study. All the cases and controls have undergone the following standard bedside cardiac autonomic function tests.

Tests for sympathetic component: 1. Lying to standing (change in systolic blood pressure),

2. Handgrip test (change in diastolic blood pressure), 3. Cold pressor test (change in diastolic blood pressure).

Tests for parasympathetic component: 1. Deep breathing test (delta heart rate), 2. Valsalva maneuver (Valsalva Ratio), 3. Lying to standing (30:15 ratio).

They were evaluated for glycemic control with FBS, PPBS and HbA1c levels. Cases were divided into sub-groups depending on duration of diabetes and glycemic control. The relation between CAN and duration of diabetes and glycemic control was determined by Statistical analysis.

Results:

60 cases with diabetes and 60 controls without diabetes were included in the study. Mean age of cases was 52.38 and controls was 51.97. There was no significant difference in age between two groups because of matching. 36 males and 24 females were included in both cases and controls.

There was no difference in sex distribution which can be attributed to matching of subjects during data collection. The mean duration of diabetes among cases was 7.28 ± 3.61 years. CAN was present in 21 (35%) of diabetic patients, 13 (21.6%) cases had early features of CAN, whereas CAN was present in 2 (3.3%) of controls and was absent in 86.6% of controls. In the study it was observed that mean age of diabetics without CAN was 48.54 ± 5.7 years, Early CAN was 52 ± 6.17 and Definitive CAN was 57.38 ± 7.2 yrs. When cases were grouped according to duration of diabetes < 5 years and >5 years, 17.6 % and 41.8% of the cases in each group respectively had CAN, which is significant. About 85.7% of the cases who had CAN have had diabetes for a duration greater than 5 years. Among cases with HbA1c levels > 7%, about 54.54% had definitive CAN and 33.3% had Early CAN. It was observed that there was significant association between increased HbA1c levels and CAN.

CONCLUSION:

The present study suggests Cardiac Autonomic Neuropathy is common in Diabetics compared to healthy individuals. Cardiac Autonomic Neuropathy is associated with increase in the age of the patient and duration of diabetes. Cardiac autonomic neuropathy is associated with poor glycemic control.

KEY WORDS: Cardiac Autonomic Neuropathy, Glycemic Control, HbA1c, Autonomic Function Tests, Type 2 Diabetes Mellitus.

LIST OF ABBREVATIONS

- ANS - Autonomic Nervous System
- ADA - American Diabetes Association
- ATP - Adenine dinucleotide Tri Phosphate
- AGE - Advanced Glycosylation End product
- ANOVA - Analysis of variance
- BP - Blood Pressure
- CAN - Cardiac Autonomic Neuropathy
- CPT - Cold Pressor Test
- CVS - Cardiovascular system
- DAN - Diabetic Autonomic Neuropathy
- DBD - Deep Breathing Difference
- DBP - Diastolic Blood Pressure
- DBT - Deep breathing test
- DCCT - Diabetes Control and Complications Trial
- DHR - Delta heart rate
- DM - Diabetes Mellitus
- DMV - Dorsal Motor nucleus of Vagus
- ECG - Electrocardiogram
- eNOS - endothelial NO synthase
- GHb - Glycosylated Hemoglobin
- HbA - Hemoglobin A
- HbA1c - Glycosylated Hemoglobin

- HGT - Hand grip test
- HR - Heart Rate
- HRV - Heart Rate Variability
- IDDM - Insulin Dependent Diabetes Mellitus
- L-NMMA - NG-monomethyl-l-arginine
- LST - Lying to Standing Test
- LVDD - Left ventricular diastolic dysfunction
- MABP - Mean Arterial Blood Pressure
- MI - Myocardial infarction
- MIBG - Meta-iodobenzylguanidine
- NA - Nucleus Ambigus
- NEFAs - Non-esterified fatty acids
- NO - Nitric oxide
- NTS - Nucleus Tractus Solitarius
- OH - Orthostatic Hypotension
- POTS - Postural tachycardia syndrome
- PTI - Postural Tachycardia Index
- P-value - Probability value
- SBP - Systolic Blood Pressure
- SD - Standard Deviation
- VLM - Ventro Lateral Medulla
- VM - Valsalva maneuver
- VR - Valsalva ratio

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INTRODUCTION

Diabetes Mellitus affects an estimated 250 million worldwide [1]. WHO reported that total diabetics in India in 2000 was 31.7 million and this number is likely to increase to 79.4 million by 2030 [2]. According to the Indian Council of Medical Research-Indian Diabetes study, a national diabetes mellitus study, India currently has 62.4 million people with diabetes mellitus [3].

Diabetes mellitus Type 2 is a metabolic disorder primarily characterized by insulin resistance, relative insulin deficiency and hyperglycemia. Complications of Diabetes are the major cause of morbidity and mortality in persons with Type 2 Diabetes mellitus (T2DM). Chronic hyperglycemia is a major initiator of micro vascular complications of Diabetes. Micro vascular complications comprise neuropathy, retinopathy and nephropathy. Neuropathy includes both peripheral and autonomic neuropathy.

Cardiac autonomic neuropathy (CAN) is one of the major complications of diabetes mellitus. It is also the most under diagnosed and least understood diabetic complications [4]. It generally manifests as exercise intolerance, resting tachycardia and orthostatic hypotension. CAN results from damage to the autonomic nerve fibers that innervate the heart and blood vessels and results in abnormalities in heart rate control and vascular dynamics [5.] CAN represents a significant cause of morbidity and mortality in diabetic patients and is associated with a high risk of cardiac arrhythmias and sudden death, possibly related to silent myocardial ischemia [6].

Poor glycemic control plays an important role in the development and progression of diabetic cardiac autonomic neuropathy, and studies have shown that reduced cardiovascular autonomic function is associated with increased morbidity and mortality [4]. Researches in this regard have suggested a strong association between hyperglycemia and the progression of micro vascular complications in diabetic patients. Glycosylated hemoglobin reflects long-term glycemic control and has proven to be a more accurate and stable measure than fasting blood glucose levels.

Clinical symptoms of autonomic neuropathy generally do not occur until long after the onset of diabetes. Subclinical autonomic dysfunction can, however, occur within a year of diagnosis in type 2 diabetes patients and within two years in type 1 diabetes patients [7].

Quantitative cardiovascular autonomic function tests are widely used to detect, verify and quantify the cardiovascular autonomic dysfunction. They have been tested for their validity and reliability [8]. These tests are performed because the procedures are straightforward, reproducible and non- invasive. Earlier studies have suggested that patients with poor glycemic control have higher prevalence of cardiovascular autonomic neuropathy than in patients with good glycemic control in type 1 diabetes[9].

Cardiac autonomic function should be assessed in most diabetic patients as it contributes to the evaluation of cardiovascular risk. The presence of CAN should lead to further awareness of possible complications and optimal control of risk factors.

Knowledge of early autonomic dysfunction can encourage patient and physician to improve metabolic control.

This study is undertaken to assess the cardiac autonomic function in patients with T2 DM and to evaluate the relationship between CAN and duration of diabetes, and glycemic control.

AIMS AND OBJECTIVES

1. To assess the cardiac autonomic function in patients with T2 DM for detection of CAN.

2. To evaluate the relationship between CAN and duration of diabetes, and glycemic control in T2 DM.

REVIEW OF LITERATURE

A) ANATOMY AND INNERVATION OF THE HEART

The heart is the center of cardiovascular system. Heart is a hollow, muscular organ that weighs between 250 – 350grams and beats over 100,000 times a day and pumps 7000 liters of blood per day through over 60,000 miles of blood vessels [10].

In human heart, SA node is located at the junction of superior vena cava with the right atrium. The AV node is located in the right posterior portion of inter-atrial septum. There are three bundles i.e, anterior internodal tract of Bachman, the middle internodal tract of Wenckebach and the posterior internodal tract of Thorel. The AV node is normally the only conducting pathway between the atria and ventricle. It is continuous with the bundle of His, which gives off left bundle branch at the top of inter-ventricular septum and continues as a right bundle branch. The left bundle branch divides into an anterior fascicle and posterior fascicle. The branches and fascicles run sub-endocardially down either side of the septum and come into contact with Purkinje system, whose fibers spread to all parts of the ventricular myocardium.

Sympathetic Nerve fibers (adrenergic nerve endings containing Norepinephrine) ramify in the heart muscle of the atria and ventricles as well as in the sinus node and the atrio-ventricular junction. Stimulation of this system speeds up the electrical pacemaker, facilitates conduction system and increases the force of heart muscle contraction. Parasympathetic fibers (cholinergic nerve endings containing Acetylcholine) ramify in the pacemaker, the atrioventicular junction and in the muscle

of two atria (there is little cholinergic innervation of the ventricles). Stimulation of this system slows the pacemaker, delays conduction through the specialized conduction system, and reduces the force of the contraction of atria [11].

Under normal resting conditions, the parasympathetic and sympathetic systems interact to regulate the heart beat at a rate of about 70 per min. Activation of sympathetic nervous system by emotion or physical stress increases the heart rate and the force of the heartbeat, thereby augmenting the output of blood from the heart. Simultaneously, sympathetic stimulation of peripheral arteries and veins also causes release of Norepinephrine into the walls of those vessels and into the circulation from the adrenal glands, thereby raising the blood pressure and increasing the return of venous blood to the heart [12].

Spinal reflex activity affects blood pressure, but the main control of blood pressure is exerted by groups of neurons in the medulla oblongata that are sometimes called collectively the Vasomotor area or Vasomotor center. Neurons that mediate increased sympathetic discharge to blood vessels and the heart project directly to sympathetic preganglionic neurons in the intermediolateral gray column (IML) of the spinal cord. One each side, the cell bodies of these neurons are located near the pial surface of the medulla in the rostral ventrolateral medulla (RVLM). Their axons course dorsally and medially and then descend in the lateral column of the spinal cord to the IML. They contain PNMT, but it appears that the excitatory transmitter they secrete is glutamate rather than epinephrine. Impulses reaching the medulla also affect the heart rate via vagal discharge to the heart. The neurons from which the vagal fibers arise are in the dorsal motor nucleus of the vagus and the nucleus ambiguus [10].

Figure 1 : AUTONOMIC NERVOUS SYSTEM

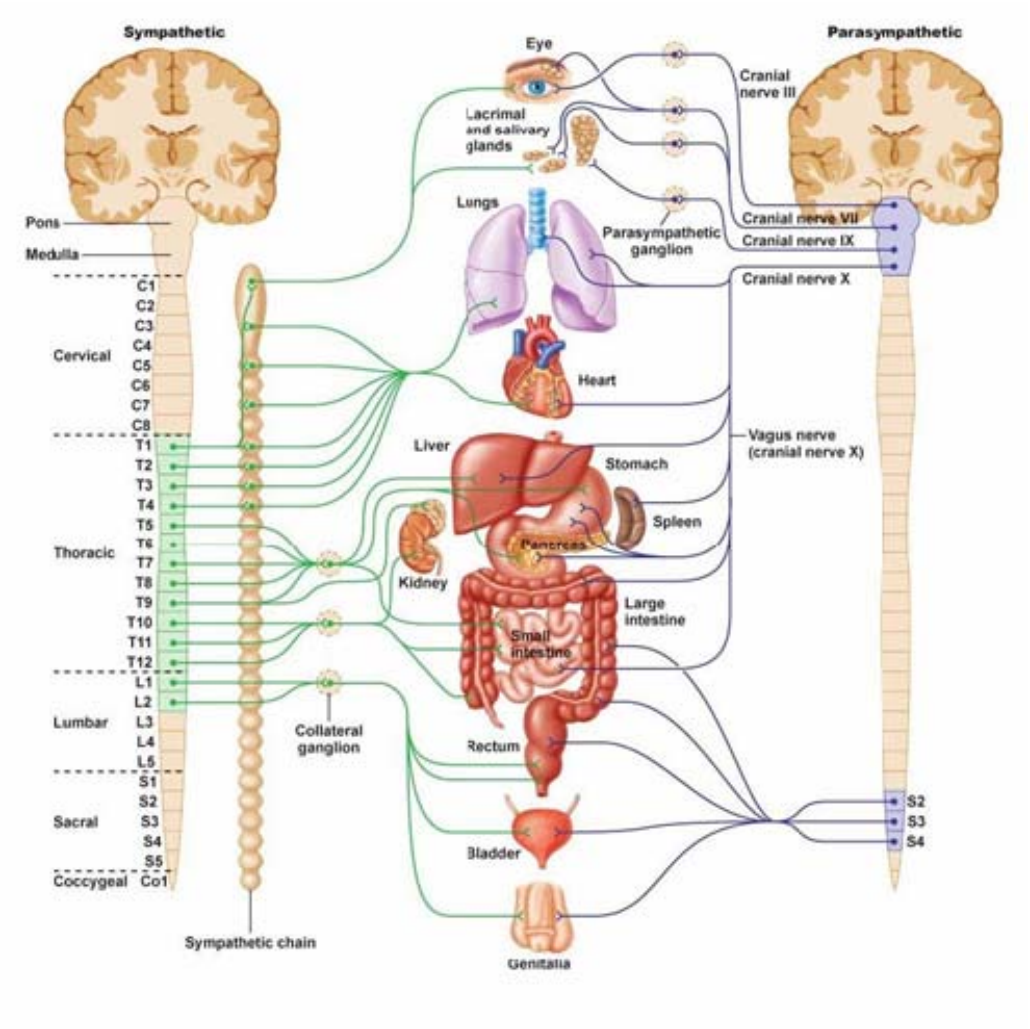
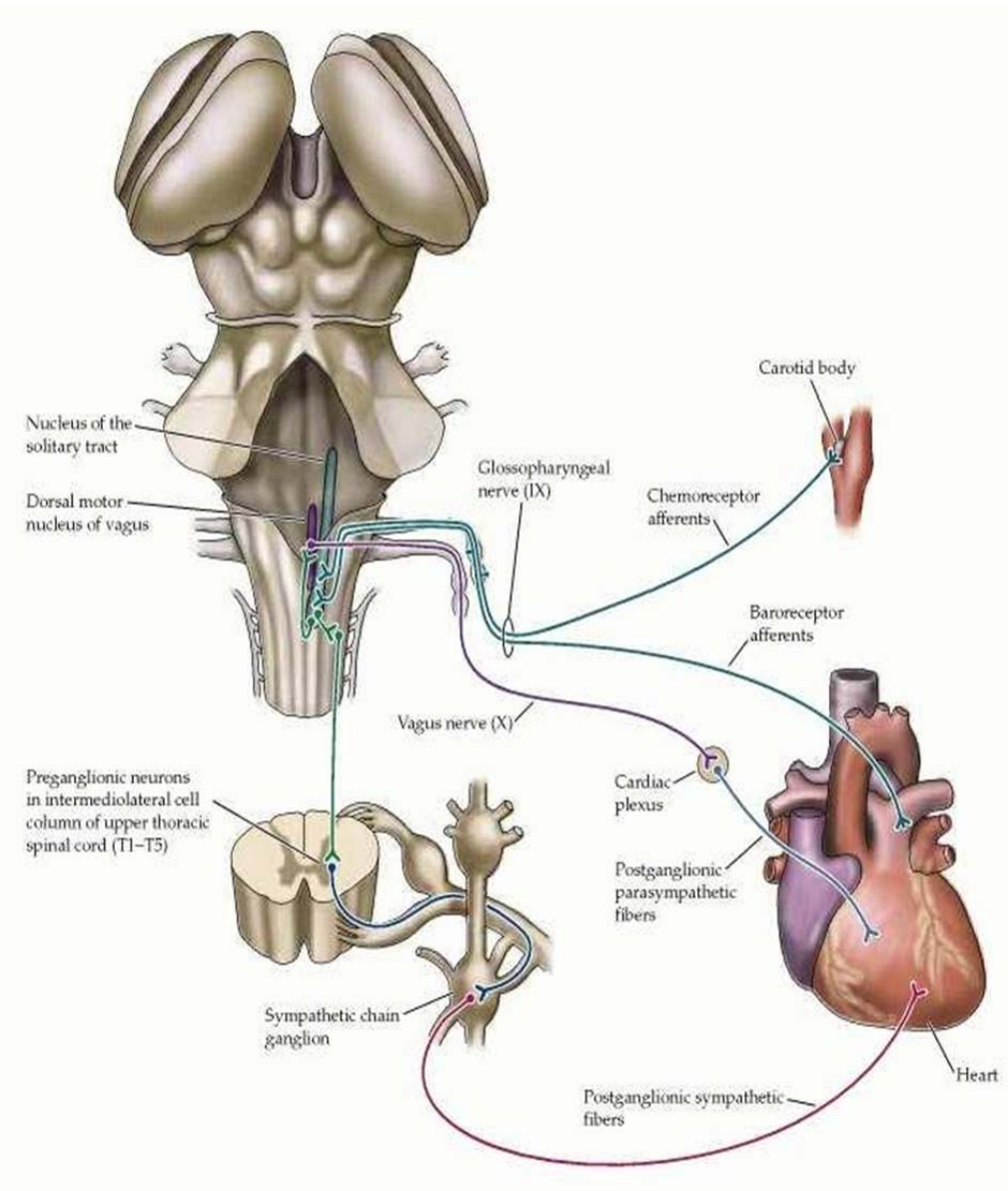


Figure 2 : AUTONOMIC INNERVATION OF THE HEART



B) AUTONOMIC REGULATION OF CARDIOVASCULAR FUNCTION

The cardiovascular system is subject to precise reflex regulation so that an appropriate supply of oxygenated blood can be reliably provided to different body tissues under a wide range of circumstances. The sensory monitoring for this critical homeostatic process entails primarily mechanical (barosensory) information about pressure in the arterial system and, secondarily, chemical (chemosensory) information about the level of oxygen and carbon dioxide in the blood. The parasympathetic and sympathetic activity relevant to cardiovascular control is determined by the information supplied by these sensors.

The mechanoreceptors (called baroreceptors) are located in the heart and major blood vessels; the chemoreceptors are located primarily in the carotid bodies, which are small, highly specialized organs located at the bifurcation of the common carotid arteries. The nerve endings in baroreceptors are activated by deformation as the elastic elements of the vessel walls expand and contract. The chemoreceptors in the carotid bodies and aorta respond directly to the partial pressure of oxygen and carbon dioxide in the blood. Both afferent systems convey their status via the vagus nerve to the nucleus of the solitary tract (Figure2), which relays this information to the hypothalamus and the relevant brainstem tegmental nuclei.

The afferent information from changes in arterial pressure and blood gas levels reflexively modulates the activity of the relevant visceral motor pathways and, ultimately, of target smooth and cardiac muscles and other more specialized structures. For example, a rise in blood pressure activates baroreceptors that, via

the pathway illustrated in Figure 2, inhibit the tonic activity of sympathetic preganglionic neurons in the spinal cord. In parallel, the pressure increase stimulates the activity of the parasympathetic preganglionic neurons in the dorsal motor nucleus of the vagus and the nucleus ambiguus that influence heart rate. The carotid chemoreceptors also have some influence, but this is a less important drive than that stemming from the baroreceptors. As a result of this shift in the balance of sympathetic and parasympathetic activity, the stimulatory noradrenergic effects of postganglionic sympathetic innervation on the cardiac pacemaker and cardiac musculature is reduced (an effect abetted by the decreased output of catecholamines from the adrenal medulla and the decreased vasoconstrictive effects of sympathetic innervation on the peripheral blood vessels).

At the same time, activation of the cholinergic parasympathetic innervation of the heart decreases the discharge rate of the cardiac pacemaker in the sino-atrial node and slows the ventricular conduction system. These parasympathetic influences are mediated by an extensive series of parasympathetic ganglia in and near the heart, which release acetylcholine onto cardiac pacemaker cells and cardiac muscle fibers. As a result of this combination of sympathetic and parasympathetic effects, heart rate and the effectiveness of the atrial and ventricular mycoardial contraction are reduced and the peripheral arterioles dilate, thus lowering the blood pressure.

In contrast to this sequence of events, a drop in blood pressure, as might occur from blood loss, has the opposite effect, inhibiting parasympathetic activity while increasing sympathetic activity. As a result, norepinephrine is released from sympathetic postganglionic terminals, increasing the rate of cardiac pacemaker

activity and enhancing cardiac contractility, at the same time increasing release of catecholamines from the adrenal medulla (which further augments these and many other sympathetic effects that enhance the response to this threatening situation). Norepinephrine released from the terminals of sympathetic ganglion cells also acts on the smooth muscles of the arterioles to increase the tone of the peripheral vessels, particularly those in the skin, subcutaneous tissues, and muscles, thus shunting blood away from these tissues to those organs where oxygen and metabolites are urgently needed to maintain function (e.g., brain, heart, and kidneys in the case of blood loss). If these reflex sympathetic responses fail to raise the blood pressure sufficiently (in which case the patient is said to be in shock), the vital functions of these organs begin to fail, often catastrophically.

A more mundane circumstance that requires a reflex autonomic response to a fall in blood pressure is standing up. Rising quickly from a prone position produces a shift of some 300–800 milliliters of blood from the thorax and abdomen to the legs, resulting in a sharp (approximately 40%) decrease in the output of the heart. The adjustment to this normally occurring drop in blood pressure (called orthostatic hypotension) must be rapid and effective, as evidenced by the dizziness sometimes experienced in this situation. Indeed, normal individuals can briefly lose consciousness as a result of blood pooling in the lower extremities, which is the usual cause of fainting among healthy individuals who must stand still for abnormally long periods (the “Beefeaters” who guard Buckingham Palace, for example).

The sympathetic innervation of the heart arises from the preganglionic neurons in the intermediolateral column of the spinal cord, extending from roughly the first through fifth thoracic segments. The primary visceral motor neurons are in

the adjacent thoracic paravertebral and prevertebral ganglia of the cardiac plexus. The parasympathetic preganglionics, as already mentioned, are in the dorsal motor nucleus of the vagus nerve and the nucleus ambiguus, projecting to parasympathetic ganglia in and around the heart and great vessels.

C) DIABETES MELLITUS

Diabetes has been known to man since centuries before Christ, Sushrutha in 5th Century B.C described it as Madhumeha - urine-resembling honey. According to Papyrus Ebers, diabetes was known 3,500 years ago in the days of Moses. Celsus was first among those who gave good clinical description and Areteaus was the first to name it "Diabetes" in the 2nd Century AD. Certain descriptions on diabetes were also observed in Chinese and Japanese writings in the early part of this century [3]. The word "Diabetes" came from a Greek word meaning, "to run through".

Thomas Willis (died I675) observed that urine of diabetes was "wonderfully sweet" and Dobson (1775) demonstrated that the sweetness was due to sugar. In 1869, Langerhans discovered the islets of pancreas, which was later given his name. In 1889 a great landmark was reached when Von Mering and Minowski produced diabetes in dogs by total pancreatomy. The pancreactomised dog survived but exhibited unexpected behavior within less than a day, frequent and voluminous urination in particular. They demonstrated that a small proportion of the gland, when implanted under the skin of a freshly depancreactised dog, prevented the appearance of hyperglycemia until the implanted tissue was removed or degenerated spontaneously [13,14].

In 1901, Opie at the John Hopkins noted that autopsies in patients who had that died of diabetes revealed glazed, shrunken and degenerated cells of Langerhans. In 1909, Jean de Mayer suggested that the pancreatic secretion that was lacking in the diabetic state to be called "Insulin" to denote its origin from the insulae of Langerhans.

In 1921, Banting and Best extracted a substance from pancreas having property of hypoglycemia in the laboratory of Professor Macleod in Toronto. This epoch making discovery opened up a new dimension in the treatment of diabetes mellitus and for which Nobel Prize was awarded to them in 1923. In January 12, 1922, Leonard Thomson became the first patient to receive Insulin at Toronto General Hospital [14].

In 1955, Franche and Fuchs used for the first time carbutamide, a sulphonyl urea derivative as an oral hypoglycemic agent, which was later banned as it caused cholangiostatic jaundice; but other suphonylureas became popular. Krall discovered biguanides as hypoglycemic agents [15,16].

Clinical features delineating autonomic neuropathy were first described in detail by Jordan in 1936 and Rundle in 1945. During 1950's and 1960's, physiologist devised a variety of techniques to ascertain the pattern of autonomic nervous involvement .The methods were cumbersome, unpleasant for the patient and highly prone for errors due to lack of patient compliance. After 1960, simple non-invasive tests were devised and it soon became apparent that autonomic involvement was not only more common than previously thought, but also that it can be detected objectively before it manifested clinically[15,16].

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia due to absolute or relative deficiency of insulin, classically associated with symptoms of excessive thirst, increased urine volume, and, if severe enough, weight loss. The fundamental defect occurs in insulin secretion and/or action. In the classical young onset form of the disorder, there is near-total insulin deficiency, with inevitable widespread metabolic changes. In the older age onset form, there is diminished and/or delayed insulin secretion in response to glucose combined with varying degrees of diminished effectiveness of circulating insulin. When there is associated obesity, insulin resistance predominates. Therefore diabetes can be defined as a state of diminished insulin action due to its decreased availability or effectiveness in varying combinations [17].

D) CARDIOVASCULAR AUTONOMIC NEUROPATHY IN DIABETES MELLITUS

Perhaps one of the most overlooked of all serious complications of diabetes is CAN [18].CAN results from damage to the autonomic nerve fibers that innervate the heart and blood vessels and results in abnormalities in heart rate control and vascular dynamics. Reduced heart rate variation is the earliest indicator of CAN [19]. Cardiovascular autonomic neuropathy (CAN), a common form of autonomic dysfunction found in patients with diabetes mellitus, causes abnormalities in heart rate control, as well as defects in central and peripheral vascular dynamics [15].

PATHOGENESIS OF CAN IN TYPE 2 DIABETES MELLITUS [6, 16.]

In diabetes, CAN is ultimately the result of complex interactions among degree of glycemic control, disease duration, age-related neuronal attrition, and systolic and diastolic blood pressure. Hyperglycemia plays the key role in the activation of various biochemical pathways related to the metabolic and/or redox state of the cell, which, in concert with impaired nerve perfusion, contribute to the development and progression of diabetic neuropathies. Experimental data implicate a number of pathogenic pathways that may impact autonomic neuronal function in diabetes including: formation of advanced glycation end products, increased oxidative/nitrosative stress with increased free radical production, activation of the polyol and protein kinase C pathways, activation of polyADP ribosylation, and activation of genes involved in neuronal damage [16].

There is strong evidence that acute physiological and pharmacological (euglycemic clamp) increments in plasma insulin concentration stimulate sympathetic activity, as determined by measurements of venous plasma catecholamines concentration , plasma NE spillover or direct microneurographic recordings of sympathetic nerve action potentials targeted at the skeletal muscle vasculature . In particular, short-term infusion of insulin and chronic hyperinsulinaemia induce a re-setting in cardiac autonomic control, mainly secondary to an increase in sympathetic activity.

These findings strengthen the hypothesis that hyperinsulinaemia/insulin resistance is implicated directly in the pathogenesis of cardiovascular mortality

associated with Type II diabetes mellitus through sustained over activation of the cardiac sympathetic nervous system. Over the past decade, evidence has been accumulated indicating a double mechanism of action of insulin: a central neural action and a peripheral action. Insulin crosses the blood–brain barrier, and insulin receptors have been found in several distinct regions of the central nervous system such as the median hypothalamus. The peripheral effects of insulin, at the cardiac sympathetic level, are mediated by NEFAs (non-esterified fatty acids) and the NO (nitric oxide)/ arginine pathway. Insulin-resistant states are characterized by alteration in both of these functions. Recent evidence indicates that these two regulatory systems interact closely and that a defect in NO synthesis and an increase in plasma NEFAs may have an important role with regard to sympathetic action. NO release accounts for the vasodilator action of insulin. *In vitro*, insulin activates l-arginine transport and stimulates NO release in cultured vascular endothelial cells. *In vivo*, insulin-induced vasodilatation is abolished by a stereospecific inhibitor of NO synthase, l-NMMA (*N*G-monomethyl-l-arginine), and by an inhibition of the synthesis of tetra-hydrobiopterin, a cofactor necessary for NO synthesis. Insulin may stimulate NO release either by a direct local effect on the vascular endothelium or by stimulating sympathetic nitrergic fibers. Comparison of vasodilatation during local intra-arterial and systemic intravenous insulin infusion has provided conflicting results.

Insulin stimulates NO release and blood flow in the denervated limb in patients who have undergone regional sympathectomy, indicating that it stimulates blood flow by a direct action at the vasculature. Consistent with this hypothesis, insulin causes hypotension in patients with autonomic failure. In innervate limbs,

however, stimulation of sympathetic vasodilator outflow by insulin appears to be necessary to induce vasodilatation, because the prevention of insulin-induced sympathetic activation by dexamethasone abolished the insulin-induced vasodilatation. The sympathetic nervous system modulates insulin-induced vasodilatation, as indicated by the much more rapid vasodilatation in the denervated compared with the innervated limb in patients with regional sympathectomy. Moreover, it is possible that there exists a balance between the central neural sympatho-excitator (via stimulation of neural peptide release) and sympatho-inhibitory (by stimulating NO release) action of insulin, as NO inhibits central neural sympathetic vasoconstrictor outflow. Thus it is possible to conclude that insulin causes vasodilatation by stimulating release of NO through a direct local effect on the vasculature and by stimulating neural vasodilator outflow. The sympathetic vasoconstrictor tone restricts insulin induced vasodilatation, and the mechanisms causing this sympathetic over activity are not known.

Hyperinsulinaemia is a candidate mechanism, but it is not invariably associated with sympathetic over activity in man, as demonstrated by the normal sympathetic nerve activity in patients with insulinoma and alternative mechanisms need be considered. NO inhibits central neural vasoconstrictor outflow in animals and humans.

It is therefore possible that the defect in NO synthesis found in many insulin-resistance states may contribute to sympathetic over activity. This defect in NO synthesis could be acquired and/or inherited. With regard to an inherited defect, recent studies indicate that polymorphisms in eNOS (endothelial NO synthase) are risk factors for CVD associated with insulin resistance.

Thus it has been hypothesized that a defect in NO synthesis could contribute to both impaired insulin induced vasodilatation and sympathetic over activity characteristic of an insulin-resistance state. More recently, the role of plasma NEFAs has also been stressed. In fact, elevated plasma NEFA levels might disrupt cardiac plasma membrane structure and function and raise intracellular calcium concentrations, thus affecting cardiac activity. It has been demonstrated that cardiac sympathetic over activity occurs in Type II diabetic patients by raising plasma NEFA concentrations. In contrast, the same group of patients submitted to intensive insulin treatment to improve metabolic control had a secondary decline in plasma NEFA levels and a decrease in cardiac sympathetic nervous system activity.

More recently, it has demonstrated that increased post-prandial plasma NEFA concentrations are associated with an enhanced degree of oxidative stress and an increased LF/HF ratio, an index of cardiac sympathovagal balance. Such data are relevant in explaining the relationship between plasma NEFAs, oxidative stress and sudden death in Type II diabetic patients. It has also been shown that increased plasma NEFA concentrations are a pro-oxidant factor.

EPIDEMIOLOGY OF CAN

Establishing the prevalence of CAN has been hampered by heterogeneous and inadequate diagnostic criteria and population selection. The prevalence of CAN varies greatly and ranges from as low as 1.6–2.6% of the primary prevention cohort in the Diabetes Control and Complications Trial (DCCT) [20], to as high as 90% of patients with longstanding type 1 diabetes who were potential candidates for a pancreas transplant [21].

In a large cohort of patients with type 1 and type 2 diabetes, Ziegler et al., using predefined criteria of HRV and spectral analysis of the beat-to-beat (R-R) intervals, found that 25.3% of patients with type 1 diabetes and 34.3% of those with type 2 diabetes had abnormal findings [22]. A recent Consensus Panel on Diabetic Neuropathy, after extensive review of the literature, concluded that the prevalence of confirmed CAN in unselected people with type 1 and type 2 diabetes is approximately 20%, but it can be as high as 65% with increasing age and diabetes duration.

Clinical correlates or risk markers for CAN are age, diabetes duration, glycemic control, micro-vascular complications (peripheral polyneuropathy, retinopathy and nephropathy), hypertension and dyslipidemia. Established risk factors for CAN are glycemic control in type 1 diabetes, and a combination of hypertension, dyslipidemia, obesity and glycemic control in type 2 diabetes [23]. Additional factors that have emerged as identifying susceptibility to cardiovascular events with intensification of glycemic control include: duration of diabetes >12–15 years, impaired renal function, coronary artery calcification, previous cardiovascular event, being African–American, being female, a history of neuropathy or numb feet and loss in HRV.

CLINICAL MANIFESTATIONS OF CAN

Traditionally, autonomic neuropathy has been considered a chronic complication that occurs only after long-term diabetes mellitus, but evidence now shows that neuropathic complications arise at least as early as the time of diagnosis of diabetes mellitus [24].

CAN is one of the most serious complications of diabetic autonomic neuropathy. It is found in one quarter of type 1 and one-third of type 2 diabetic patients, is associated with increased mortality and silent myocardial ischemia, and might even predict the development of stroke. CAN might even have greater predictive power than traditional risk factors for cardiovascular events. CAN has been linked to resting tachycardia, postural hypotension, exercise intolerance, enhanced intraoperative or perioperative cardiovascular liability, increased incidence of asymptomatic ischemia, myocardial infarction and decreased rate of survival after myocardial infarction. Cardiac autonomic dysfunction can affect daily activities of individuals with diabetes, might affect their quality of life and might invoke potentially life-threatening outcomes [25].

Resting Tachycardia.

Whereas abnormalities in HRV are early findings of CAN, resting tachycardia and a fixed heart rate are characteristic late findings in diabetic patients with vagal impairment. Resting heart rates of 90 to 100 bpm and occasional heart rate increments up to 130 bpm occur. The highest resting heart rates have been found in patients with parasympathetic damage, occurring earlier in the course of CAN than sympathetic nerve function; in those with evidence for combined vagal and sympathetic involvement, the rate returns toward normal but remains elevated [26]. A fixed heart rate

that is unresponsive to moderate exercise, stress, or sleep indicates almost complete cardiac denervation. Thus, heart rate may not provide a reliable diagnostic criterion of CAN in the absence of other causes unless it is increased by more than 100 bpm[25].

Exercise Intolerance.

Autonomic dysfunction impairs exercise tolerance, reduces response in heart rate and BP, and blunts increases in cardiac output in response to exercise. Diabetic patients who are likely to have CAN should be tested for cardiac stress before undertaking an exercise program. Patients with CAN need to rely on their perceived exertion, not heart rate, to avoid hazardous levels of intensity of exercise [27].

Orthostatic Hypotension.

Orthostatic hypotension is defined as a fall in BP (i.e., >20 mm Hg systolic or >10 mm Hg diastolic BP) in response to a postural change from supine to standing [28]. Symptoms include weakness, faintness, dizziness, visual impairment, and even syncope after a change from a lying to a standing posture. Orthostatic hypotension may become disabling, but the BP fall may also be asymptomatic [29].

Intraoperative and Perioperative Cardiovascular Instability.

Perioperative cardiovascular morbidity and mortality are increased 2- to 3-fold in patients with diabetes. Compared with non-diabetic subjects, diabetic patients undergoing general anesthesia may experience a greater degree of decline in heart rate and BP during induction of anesthesia and less of an increase after tracheal intubation and extubation.

Vasopressor support is needed more often in diabetic individuals with CAN

than in those without CAN [30]. The normal autonomic response of vasoconstriction and tachycardia does not completely compensate for the vasodilating effects of anesthesia. There is an association between CAN and more severe intraoperative hypothermia[31] that results in decreased drug metabolism and impaired wound healing. Reduced hypoxic-induced ventilatory drive[32] requires preoperative CAN screening for loss of HRV. The anesthesiologist and surgeon should be alerted to this risk.

Silent Myocardial Ischemia/Cardiac Denervation Syndrome.

Reduced appreciation for ischemic pain can impair timely recognition of myocardial ischemia or infarction, thereby delaying appropriate therapy. In the ECGs of diabetic patients with exertional chest pain, a prolonged anginal perceptual threshold (i.e., the time from onset of 0.1 mV ST depression to the onset of angina pectoris during exercise) was associated with the presence of CAN[33]. Hence, patients with CAN and coronary artery disease are jeopardized because the longer threshold permits them to continue exercising despite increasing ischemia.

Silent ischemia in diabetic patients may either result from CAN, from autonomic dysfunction attributable to coronary artery disease itself, or from both. In the Framingham Study, the rates of unrecognized MIs were 39% in diabetic patients and 22% in non-diabetic subjects, but the difference was not significant[34].

In a survey from the National Registry of Myocardial Infarction 2 (NRMI-2), of 434 877 patients presenting with MI, 33% did not have chest pain. Among those

presenting without chest pain, 32% had diabetes versus 25.4% in the group with chest pain[35].

The mechanisms of painless myocardial ischemia are, however, complex and not fully understood. Altered pain thresholds, sub threshold ischemia not sufficient to induce pain, and dysfunction of the afferent cardiac autonomic nerve fibers have all been suggested as possible mechanisms[36].

Positron emission tomography to measure regional cerebral blood flow as an index of regional neuronal activation has shown that impaired afferent signaling resulting from autonomic dysfunction is associated with failed signal transmission from the thalamus to the frontal cortex[37].

In the Detection of Ischemia in Asymptomatic Diabetics (DIAD) study of 1123 patients with type 2 diabetes, cardiac autonomic dysfunction was a strong predictor of ischemia[38]. Thus,patients with CAN warrant more careful attention, and cardiovascular autonomic function testing may be an important component in the risk assessment of diabetic patients with coronary artery disease. Given the complex and controversial mechanisms of silent myocardial ischemia, even in the absence of diabetes, further studies are needed to clarify the exact role of CAN in this context.

Features of a MI in patients with CAN are silence, cough, nausea and vomiting, dyspnea, tiredness, and electrocardiogram (ECG) changes. Reduced appreciation for ischemic pain can impair timely recognition of myocardial ischemia or infarction, thereby delaying appropriate therapy. Thus, patients with CAN warrant

more careful attention and cardiovascular autonomic function testing might be an important component in the risk assessment of diabetic patients with coronary artery disease.

Orthostatic Tachycardia and Bradycardia Syndromes.

Symptoms compatible with orthostasis, such as feeling faint or dizzy, circumoral paresthesia, and headache, may occur on changes from a supine to an erect position and may be caused by postural tachycardia syndrome (POTS), inappropriate sinus tachycardia, neurocardiogenic syncope, or abnormalities in baroreceptor function. The hallmark of these abnormalities is the absence of a fall in BP with standing, but a tachycardia or bradycardia with the change in posture. The pathogenesis of POTS is obscure. Some patients have defective peripheral vasoconstriction and an increase in calf blood flow, whereas others have increased peripheral arterial resistance and decreased blood flow [39,40].

POTS is associated with a selective defect in intra-epidermal nerve fiber in the skin. Norepinephrine concentrations have been significantly related to the estimate of the severity of autonomic neuropathy [40] and loss of peripheral sympathetic C fiber tone seems to translate to inadequate cardiac venous return with thoracic hypovolemia [39].POTS patients have paradoxically unchanged plasma renin activity and low aldosterone, given their marked reduction in plasma volume. These patients also have a significant reduction in plasma erythropoietin, suggesting that the kidney may play a role in the pathogenesis of this condition.

POTS patients have exaggerated muscle sympathetic nerve activity with baroreceptor-reflex challenges [41]. A cadre of POTS patients have shown normal peripheral resistance and blood volume in the supine position but thoracic hypovolemia and splanchnic pooling in the upright position. Selective and maintained orthostatic pooling in the splanchnic bed occurs in low-flow POTS despite marked peripheral vasoconstriction in these patients. Local splanchnic vasoregulatory factors may counteract the vasoconstriction in these patients [42]. In addition to these syndromes, there are selected patients with orthostatic symptoms who have a paradoxical bradycardia on standing; the symptoms closely mimic those of hypotension. It is important to recognize these differences because each is amenable to simple intervention.

Autonomic Cardiomyopathy.

CAN might be associated with abnormalities in left ventricle (LV) systolic and, particularly, diastolic functions in the absence of cardiac disease in diabetic patients. Studies have shown a significant correlation of the severity of CAN with reduced peak diastolic filling rate and with an augmented atrial contribution to diastolic filling as assessed by Doppler echocardiography. It is difficult to judge, however, whether CAN is an independent contributor to these abnormalities, because other factors, such as interstitial myocardial fibrosis and microangiopathic or metabolic changes, might also be responsible for LV dysfunction.

CAN has been associated with LV diastolic dysfunction (LVDD) at rest, both in patients with long-term type 2 [43] or type 1 diabetes [44]. LVDD can progress to heart

failure, mainly with preserved LV systolic function (diastolic heart failure), which is also related to high morbidity and mortality rates [45,46]. The pathophysiology of LVDD includes delayed relaxation, impaired LV filling and/or increased stiffness [47]. In patients with CAN, vagal impairment can lead to a relative predominance of sympathetic activity in the sympathovagal balance. Sympathetic overactivity stimulates the renin–angiotensin–aldosterone system and increases heart rate, stroke volume and peripheral vascular resistance, thus contributing to LV dysfunction [48].

Association of CAN with Major Cardiovascular Events

The relationship between CAN and major cardiovascular events has been assessed in 2 prospective studies [49]. Specifically, the relationship between baseline CAN and the subsequent incidence of a fatal or nonfatal cardiovascular event, defined as an MI, heart failure, resuscitation from ventricular tachycardia or fibrillation, angina, or need for coronary revascularization, was examined [49]. The relative risks associated with CAN in these studies were 2.2 and 3.4, respectively, with the latter result just achieving statistical significance ($P<0.05$). There seems to be an association between CAN and major cardiovascular events, but given the small number of events that occurred in each of these studies, more follow-up studies are required.

Sudden Death in CAN

Sudden, unexpected deaths occur among subjects with CAN. One potential cause may be severe but asymptomatic ischemia, which can induce lethal arrhythmias. QT prolongation may also predispose individuals to life-threatening

cardiac arrhythmias and sudden death. Results from the European Diabetes Insulin-Dependent Diabetes Mellitus (IDDM) Complications Study showed that male patients with impaired HRV had a higher corrected QT prolongation than males without this complication [50].

Imaging of myocardial sympathetic innervation with various radiotracers (e.g., MIBG) has shown that predisposition to arrhythmias and an association with mortality may also be related to intracardiac sympathetic imbalance [51,52].

CAN and Cerebrovascular Disease

Abnormalities of parasympathetic and sympathetic autonomic function were found to be independent predictors of stroke in a group of 133 type 2 diabetic patients for 10 years [53]. Clearly, other studies examining all the multivariate factors contributing to stroke are needed to confirm or refute this report.

Increased Mortality after MI

Mortality rates after an MI are higher for diabetic compared with non-diabetic patients [54]. A simple bedside test that measured 1-minute HRV during deep breathing was a good predictor of all-cause mortality for 185 patients (17.8% with diabetes) after a first MI [55]. Autonomic function testing is a valuable tool in identifying a subgroup of post-MI patients who are at high risk for death.

E) AUTONOMIC FUNCTION TESTS IN CAN

The functional characteristics of the autonomic nervous system can be assessed by physiologic and pharmacologic tests. Commonly used physiologic tests primarily assess autonomic aspects of cardiovascular function. These tests are non-invasive, easy to use, and provide quantitative or regional information about autonomic function. Interpretation of results requires collection of data under controlled circumstances. Pharmacologic tests can elucidate pathophysiological abnormalities and guide the development of rational therapy.

There is no widely accepted single approach to the diagnosis of CAN in diabetes. Assessment of HRV, orthostatic hypotension, and 24-h blood pressure profiles provides indexes of both parasympathetic and sympathetic autonomic function and can be used in clinical settings. Other methods such as cardiac sympathetic imaging, microneurography, occlusion plethysmography, and baroreflex sensitivity are currently used predominantly in research settings but may find a place in the clinical assessment of CAN in the future.

Testing autonomic function is complicated by the fact that within each part of the output from the autonomic system partial responses occur and any defects, whether central or peripheral, may be partially corrected by other neuronal, chemical, or hormonal mechanisms. When lesions caused by disease is added, the task of correlating reflex defects and pathology might seem daunting. However, the cardiovascular system has proved suitable for an analysis of the principles used in testing for autonomic dysfunction [56].

The clinical evaluation of autonomic dysfunction has proved laborious due to large number of individual variations in the autonomic nervous system functions. The major aim of investigations has been to determine the normality of autonomic function, to assess the degree of autonomic dysfunction, and to ascertain whether any abnormality is primary or secondary. A range of investigations has been conducted, with the emphasis on the cardiovascular system, and there have been numerous advances, especially in the field of non- invasive measurements. The information of each test should be considered in relation to the clinical picture as a whole, since the assessment is dependent not only on the reflex arcs and afferent nerve activity, but also on end organ responsiveness and the individual characteristics of subjects [57].

Today, sensitivity and early assessment of cardiovascular autonomic neuropathy is possible by means of noninvasive autonomic function tests, including time domain (statistical analysis), indices of heart rate variability, aiming at prevention of advanced stages.

However a generally accepted standardization of the various test procedure is needed. Despite this problem, it is estimated that cardiovascular autonomic neuropathy can be detected by abnormal autonomic function tests in at least one quarter of Type – I and one third of Type – II diabetic patients. In some cases, autonomic dysfunction may be present at the time of manifestation of both Type-I and Type – II diabetes.

There is increasing evidence suggesting that the statistical, geometric, frequency domain and non-linear measure of 24 hour heart rate variability could be more sensitive and reliable in detecting cardiovascular autonomic neuropathy when compared with autonomic function tests [58]

Standard tests of cardiac autonomic function were initially used to classify subjects according to the presence or absence of neuropathy; however, more recent studies attempted to grade the severity of neuropathy[59].

During the 1970s, Ewing et al.[60] devised a number of simple bedside tests of short-term R-R differences to detect CAN in diabetic patients, including: changes in R-R with deep breathing, which measures sinus arrhythmia during quiet respiration and primarily reflects parasympathetic function; R-R response to standing, which induces reflex tachycardia followed by bradycardia and is jointly vagal and baroreflex mediated; and Valsalva ratio, which evaluates cardiovagal function in response to a standardized increase in intrathoracic pressure (Valsalva maneuver), primarily parasympathetic mediated.

Autonomic Function Tests.

1) **Blood Pressure response to standing:**

Difference in systolic blood pressure between lying and after standing for 1 minute.

Normal : ≤ 10 mmHg. Borderline : 11 – 20 mmHg. Abnormal : ≥ 30 mmHg [61] .

2) **Blood Pressure response to sustained handgrip:**

Take resting systolic blood pressure. Maintain handgrip in other arm at 30 % of maximum voluntary pressure for up to 5 minutes; record systolic pressure each minute. Stop if rise reaches normal level. If not, record rise to just before handgrip release at 5 minutes.

Normal: ≥ 16 mmHg. Borderline: 11 – 15 mmHg. Abnormal: ≤ 10 mmHg [61].

3) **Blood Pressure response to cold pressor test:**

Immersion of hand into cold water causes an activation of the sympathetic nervous system as evidenced by the marked increase in blood pressure and heart rate[62]. The cold pressor test is evaluated by immersion of subjects left hand (up to wrist) in cold water at 8 ^{0}C for 2 min. in recumbent state. Blood pressure is measured before immersion and 1 min. after immersion of hand [63]. In normal persons immersing of hands in ice water raises the systolic pressure by 15 – 20 mmHg and the diastolic pressure by 10 – 15 mmHg [64]. Changes in the blood pressure (BP) response to a 1-min immersion of the hand into ice water, the cold pressor test (CPT). An about 8 mmHg BP increase in the CPT reported by others for health and an elevation >25 mmHg for patient with "hypertension" have been viewed as predisease [65].

4) Heart Rate response to Standing:

Attach ECG limb leads. With strip recorder running in lead II, subject/patient stands from lying as quickly as possible. Measure 30: 15 ratio i.e. ratio of longest R-R interval around 30^{th} beat after standing to shortest R-R interval about 15^{th} beats after standing.

Normal: ≥ 1.04. Borderline: 1.01-1.04. Abnormal: ≤ 1.00 [61].

5) Heart rate response to Deep Breathing:

With patient sitting and strip ECG recording, patient breaths deeply and evenly at 6 breaths per minute (5 sec. in, 5 sec. out) for 3 cycles (30sec.). Count in 2-3-4-5-out-2-3-4-5-as they do. Measure greatest heart rate difference during each cycle and average the 3 differences.

Normal: ≥ 15 beats / min. Borderline: 11 – 14 beats / min. Abnormal: ≤ 10 beats / min[61].

6) Heart rate response to Valsalva maneuver:

The quantitative Valsalva maneuver is performed by blowing with open glottis into a mouth piece connected to mercury column of a sphygmomanometer with air leak. A 40–50 mmHg. Pressure is maintained for 15 sec. BP recovery in phase II and cardiopressor response in phase IV are indices of vasoconstrictor and contractile integrity. Baroreceptor mediated tachycardia in phase II and bradycardia in phase IV determine if cardiovagal reflexes are intact. The tachycardia ratio (ratio of shortest R-

R interval during the test to the longest R-R interval before the test) and the Valsalva ratio (ratio of longest R-R interval after the maneuver divided by the shortest R-R interval during the test) [66,67].

Normal - >1.21. Borderline - 1.11 – 1.20. Abnormal <1.10.

Scoring for Autonomic Function Tests[67].

Scoring for parasympathetic and sympathetic components.

Normal = all test normal or one test borderline.

Early = one test abnormal or two test borderline.

Definite = two tests abnormal.

These validated tests, described in detail in a statement by the American Diabetes Association [68], are recommended for CAN diagnosis [69] and can be performed in the practitioner's office.

7) Head Up Tilt Table Testing

The rapid postural changes that are part of head-up-tilt-table testing, with/without pharmacological provocation, can be used for the investigation of CAN or of predisposition to neurally mediated (vasovagal) syncope due to the wide range of changes in the autonomic input to the heart and in the R-R intervals. This test requires specialized personnel and is not readily available in general practice. Tilt table testing is done to evaluate neurally mediated hypotension, an excessive increase in pulse rate (POTS) or fainting (neurally mediated syncope).

The patient lies on a stretcher-like support. Straps that are similar to seatbelts are attached around the abdomen and legs and the patient is tilted upright. The exact angle of the tilt varies and is usually between 60 and 80 degrees. The tilting goes on for up to 45 minutes. The patient is gradually tilted to an upright position until systolic blood pressure drops to 70 mm Hg or the appearance of orthostatic symptoms such as dizziness, lightheadedness or faintness. The purpose is to hopefully reproduce the patient's problem in a controlled laboratory setting. It may not be performed on all patients, such as patients with a persistent fall in blood pressure each time they stand up (orthostatic hypotension) because the blood pressure will fall progressively beginning as soon as the tilting starts.

More recently, focus has shifted to lesser degrees of adrenergic failure and patients with vasodepressor syncope. To evaluate these patients, the duration of tilt has been extended to 60 minutes and infusions of isoproterenol have been given. Isoproterenol is given to induce vasodepressor presyncope and evaluate receptor supersensitivity and the presence of autonomic failure. A tilt test for 40 minutes without isoproterenol infusion has been suggested to be adequate in separating patients with and without vasodepressor syncope; isoproterenol infusion should be avoided because it degrades the specificity of the test.

Figure 3: Patient undergoing HEAD-UP-TILT-TEST.

8) Spectral analysis of HRV

Spectral analysis of HRV is another tool to evaluate CAN. It decomposes the R-R signal into a set of sine and cosine waves and estimates the magnitude of variability as a function of frequency. The main frequency components described are very-low-frequency components (<0.04 Hz) related to fluctuations in vasomotor tone associated with thermoregulation, the low-frequency component (0.04–0.15 Hz) associated with the baroreceptor reflex, and the high-frequency components (0.15–0.4 Hz) related to respiratory activity. It is generally thought that the sympathetic system modulates the lower-frequency HRV components, whereas the parasympathetic system controls the high-frequency HRV components. Different mathematical methods have been used to analyze HRV. Fourier transform is the most commonly chosen due to algorithm simplicity and high processing speed. This method, limited to stationary signals, is based on the assumption of steady-state conditions discarding any dynamics in the power spectrum and does not allow a precise detection of a sudden change in autonomous tone or a precise localization of a particular event in time when examining non-stationary conditions. The continuous wavelet transform equations perform a time-frequency decomposition of the signal yielding a time dependent version of the typical low- and high-frequency peaks. Commercially available software programs using these methods are available for assessment of HRV.

Imaging Techniques for CAN [70,71,72.]

Quantitative scintigraphic assessment of sympathetic innervation of the human heart is possible with positron emission tomography (PET) and either [^{123}I] *meta*-iodobenzylguanidine (MIBG) or [^{11}C]-*Meta*-hydroxy-ephedrine ([^{11}C] HED) [70]. Deficits of LV [^{123}I] MIBG and [^{11}C] HED retention have been identified in type 1 and type 2 diabetic subjects with and without abnormal cardiovascular reflex testing [71].

Metabolically stable [^{11}C] HED undergoes highly specific uptake into sympathetic nerve varicosities via norepinephrine transporters, and quantitative [^{11}C] HED retention may be assessed in 480 independent LV regions. The striking consistency of the evolution of the pattern of denervation in type 1 diabetes supports the reliability of [^{11}C] HED to monitor changes in cardiac sympathetic nerve populations and evaluate early anatomical regional deficits of sympathetic denervation. Quantitative regional measurements of myocardial β-adrenoreceptor density can also be assessed using PET and the high-affinity β-adrenoreceptor radioligand [11C] CGP-12177.

However, postsynaptic β-adrenoreceptor density was never assessed in human diabetes.

Baroreflex Sensitivity [73]

Baroreflex sensitivity (BRS) is a technique that evaluates the capability to reflexively increase vagal activity and decrease sympathetic activity in response to a sudden increase in blood pressure. It is used in research protocols to assess cardiac vagal and sympathetic baroreflex function and is calculated from the measurement of the heart rate–blood pressure relation after an intravenous bolus of phenylephrine. The BRS was a significant independent risk predictor of cardiac mortality in the Autonomic Tone and Reflexes after Myocardial Infarction (ATRAMI) study, a large international multicenter prospective study of 1,284 patients with a recent myocardial infarction. It has been shown that the analysis of spontaneous baroreflex sequences gives results equivalent to the pharmacological methods, which lead to development of techniques based on servoplethysmomanometry that measures blood pressure in the finger on a beat-to-beat basis (Finapress).

Microneurography [73]

This technique is based on recording electrical activity emitted by peroneal, tibial, or radial muscle sympathetic nerves and identification of sympathetic bursts. Bursts have a characteristic shape consisting of a gradual rise and fall that is usually constrained by the cardiac cycle and at least twice the amplitude of random fluctuations. Recently available fully automated sympathetic neurogram techniques provide a rapid and objective method that is minimally affected by signal quality and preserves beat-by-beat sympathetic neurograms.

Treatment of CAN

For the abnormalities in autonomic balance, adrenergic excess can be addressed with beta blockers and parasympathetic excess with anticholinergic drugs [74]. Intervention studies have documented the protective effects of glycemic control on autonomic function in type 1 diabetic patients (DCCT trial).

In the Steno memorial trial, Gaede et al. [75,76] showed that a multifactorial strategy aimed at lifestyle change with pharmacological correction of hyperglycemia, hypertension, dyslipidemia and microalbuminuria in type 2 diabetic patients reduces abnormalities in autonomic function by 68%.

Ziegler et al. [77] reported that alpha lipoic acid improves autonomic function. In fact, it is one of the only drugs targeting the nervous system to be endorsed by the Toronto Consensus [78]. In contrast, failure to identify loss of parasympathetic integrity is accompanied by dire consequences, as witnessed by the 22% increase in sudden death in the ACCORD study with intensification of glucose control [79].

The Toronto Consensus Panel on Diabetic Neuropathy[78] concluded the following in relation to CAN treatment:

- Intensive diabetes therapy retards the development of CAN in type 1 diabetes (level A).
- Intensive multifactorial cardiovascular risk intervention retards the development and progression of CAN in type 2 diabetes (level B).
- Lifestyle intervention might improve HRV in prediabetes (level B) and diabetes (level B).
- Symptomatic orthostatic hypotension might be improved by non-pharmacological measures (level B), and by midodrine (level A) and/or fludrocortisone (level B).

The recommendations from the Toronto Consensus Panel on Diabetic Neuropathy[78] are as follows:

- Diabetes therapy in patients with type 1 and type 2 diabetes should consider the individual risk profile and comorbidities (class I).
- Lifestyle intervention should be offered as a basic preventive measure (class I).
- Given the limited evidence from very few large-scale randomized clinical trials, recommendations cannot be given for pharmacological and non-pharmacological treatments of CAN.
- Drugs that might reduce HRV should be avoided in patients with CAN (class III)

- Resting tachycardia associated with CAN can be treated with cardioselective beta-blockers (class I).
- The first therapeutic approach in symptomatic orthostatic hypotension should consider the exclusion of drugs exacerbating orthostatic hypotension, correction of volume depletion (class I) and other non-pharmacological measures (class II a).
- Pharmacotherapy of symptomatic orthostatic hypotension should include midodrine (class I) or fludrocortisones, or a combination of both in non-responders to monotherapy (class II a).
- Because of the limited evidence, the potential risk of any pharmacological treatment should be thoroughly weighed against its possible benefit (class I).

REVIEW OF STUDIES ON CAN

A study by Bhatia S.G, Sainani G.S, Nayak N.J. & Diwate P.G. (1976) on Valsalva maneuver as a test of autonomic neuropathy in random group of 100 patients of diabetes mellitus for clinical evidence of autonomic and peripheral neuropathy, 26 had clinical evidence of autonomic neuropathy of which 21 cases showed abnormal valsalva response. Among the remaining 74 cases, who did not have clinical evidence of autonomic neuropathy, only 3 showed abnormal response. There was also significant association between occurrence of autonomic and peripheral neuropathy[80] .

Ewings D.J, Campbell I. W and Clarke B. F in 1981 in their study on heart rate changes in diabetes mellitus, of the 61 diabetics, 22 had normal and 14 abnormal parasympathetic function and 22 had abnormal parasympathetic + sympathetic function. There were no significant difference in mean age between three groups i.e.

Group – I : diabetics showing normal response (49 years), Group–II : diabetics showing abnormal response to parasympathetic functions (53 years) and

Group–III : diabetics showing abnormal response to parasympathetic + sympathetic functions (47 years) [81].

In a study done on "Diabetic Autonomic Neuropathy and Cardiovascular risk" Pittsburgh Epidemiology of Diabetes complications study III, Diabetic autonomic Neuropathy (DAN) has been shown to confer a high risk of mortality. The association between Diabetic autonomic Neuropathy (DAN) and cardiovascular risk factors was examined in a well-defined cohort of 25 to 34 year old insulin dependent diabetes Mellitus subjects (n=168) with and without DAN as evaluated by heart rate response to deep breathing, standing and the valsalva manoeuver. These results suggest that traditional cardiovascular risk factors are important correlates of diabetic autonomic neuropathy and may relate to both cause and poor prognosis [82].

Selvin and colleagues performed a careful meta-analysis of 10 cohort studies involving 7435 people with type 2 diabetes and 3 cohort studies involving 1688 people with type1diabetes. For type 2 diabetes, a 1% point absolute increase in glycosylated hemoglobin was associated with a significant 18% (95% CI, 10% to 26%) increase in the risk for coronary heart disease or stroke and a 28% increase in the risk for peripheral vascular disease. A similar but nonsignificant relationship was noted for type 1 diabetes. These data highlight the utility of the glycosylated hemoglobin level as a measure of risk for cardiovascular events in type 2 diabetes and suggest that it may also reflect cardiovascular risk in people with type 1 diabetes [83].

Khaw and colleagues carefully analyzed the relationship of hemoglobin A1c measurement to incident cardiovascular events in a 6-year cohort study of 10,232 diabetic and nondiabetic men and women age 45 to 79 years. Finally, they noted very small shifts in the general population's average hemoglobin A1c level (for example, 0.1% to 0.2%) could dramatically affect the future incidence of cardiovascular disease [84]. Over 10 years, hemoglobinA1c (HbA1c) was 7.0% (6.2-8.2) in the intensive group compared with 7.9% (6.9-8.8) in the conventional group an 11% reduction. There was no difference in HbA1c among agents in the intensive group. Compared with the conventional group, the risk in the intensive group was 12% lower (95% CI 1-21, p=0.029) for any diabetes-related endpoint, 10% lower (11 to 27, p=0.34) for any diabetes-related death and 6% lower (10 to 20, p=0.44) for all-cause mortality. This concluded that intensive blood-glucose control by either sulphonylureas or insulin substantially decreases the risk of microvascular complications [85].

A study by Ghosh Asutosh and Mukherjee Chandra Subhas (1998) on evaluation of autonomic function in diabetes mellitus in 30 diabetic patients along with 20 controls, commonest classical symptoms of diabetes was weight loss and commonest autonomic symptom was giddiness. Symptoms of autonomic neuropathy were giddiness (36.66%), impotence in male (26.66%), bladder dysfunction (20%), diarrhoea (13.33%). Laboratory abnormalities suggestive of autonomic neuropathy were postural hypotension (26.66% in male, 33.34% in female). Abnormal valsalva ratio (53.34% in male, 33.34% in female), abnormal 30:15 beat ratio (53.33% in male, 46.66% in female), abnormal expiratory/inspiratory ratio (80.02% in male, 73.36% in female). Cold pressor test was normal. Combined autonomic and peripheral

neuropathy was found in 37.5% cases of IDDM and 22.72% cases of NIDDM. Only autonomic neuropathy was found in 62.5% cases of IDDM and 45,45% cases of NIDDM [86].

The longer the duration of diabetes, the more likely is the occurrence of hyperglycemic states even in adequately controlled diabetic. The positive correlation between duration of diabetes type II and autonomic neuropathy has been reported by Philips JC[87] , Ninkovic[88], Valensi[89].

In 2006 Ling DY et al studied risk factors for diabetic cardiovascular autonomic neuropathy (CAN) in patients with type 2 diabetes to establish a regression model for evaluating the diagnosis of CAN. 325 patients with type 2 diabetes were divided into four groups according to the results of four standard function tests. Detailed disease history, physical examination of every patient, serum and urine tests were done. 64.0% of the patients were found to have abnormal autonomic function and 30.2% definite CAN. There was significant difference among the groups about age, average glycosylated form of hemoglobin (HbA1c). The incidence of diabetic complications and accompanying diseases increased with deterioration of CAN ($P < 0.05$). The regression model showed that age, average HbA1c, hypertension, peripheral neuropathy, retinopathy, tachycardia at rest and duration of peripheral neuropathy were significant related factors for CAN, and concluded that except for age and hypertension, risk factors were all induced by hyperglycemia. It is suggested that control of hyperglycemia is of primary importance in preventing diabetic complications [90].

In a 7 year follow-up study published by Ko SH et al, in 2008, the development of cardiovascular autonomic dysfunction was higher in older patients (P<0.001); in those with longer duration of diabetes (P<0.001); of hypertension (P = 0.005), and of diabetic retinopathy (P<0.001); and in those who had higher levels of microalbuminuria (P = 0.002) [91].

In 2013, Rodica Pop-Busui et al, published a study in which CAN is associated with increased LV mass and concentric remodeling as assessed by Cardiac MRI independent of age, sex, and other factors [92].

Cardiovascular Autonomic Dysfunction predicted Severe Hypoglycemia in Patients with Type 2 Diabetes in a 10 year follow-up study by Jae-Seung Yun [93]. In a 2013 study by M Charles et al to evaluate Impact of early detection and treatment of diabetes on the 6 year prevalence of Cardiac Autonomic Neuropathy in people with screen – detected Diabetes, 190 general practices were randomized to deliver either intensive treatment (IT) or routine care (RC) to T2 DM patients. Prevalence of early CAN was 15.1% in RC group and 15.5% percent in IT group. While manifest CAN was 7.1% and 7.3% respectively. No statistical significant effect of intensive treatment of prevalence of CAN [94].

In 2013, Didangelos T studied the effect of ACE inhibition or angiotensin receptor blockade and their combination on both CAN and LVD in asymptomatic patients with diabetes. Early ACE inhibition or angiotensin receptor blockade improved both CAN and LVDD after 1 year of treatment in asymptomatic patients

with type 1 or 2 diabetes mellitus. The combination was slightly better than monotherapies on CAN and LVDD [95].

In a 2014 study by Tarvainen MP et al, HRV parameters showed a clear difference between healthy controls and T2DM patients. Hyperglycemia was associated with increase in mean heart rate and decrease in HRV, indicated by negative correlations of BGL and HbA1c with mean RR interval and most of the HRV parameters. Duration of diabetes was strongly associated with decrease in HRV, the most significant decrease in HRV was found within the first 5-10 years of the disease [96].

A study by Cardoso CR in 2014 to evaluate " Relationships between reduced heart rate variability and pre-clinical cardiovascular disease in patients with type 2 diabetes" it was found patients with reduced HRV had longer diabetes duration, greater prevalences of microvascular complications, lower physical fitness, and higher heart rate, glycated hemoglobin, albuminuria and LVMI than patients with normal HRV [97].

A 2014 large-scale, population-based, cross-sectional study to explore the relationships of CAN with DM and resting HR by Tang ZH et al, shows DM and resting HR were very significantly and independently associated with CAN ($P < 0.001$ for both). Resting HR alone or combined with DM (DM-HR) both strongly predicted CAN [98].

A 2014 study by Tahrani AA et al, to assess the impact of cardiac autonomic neuropathy (CAN) on the development and progression of chronic kidney disease (CKD) in patients with type 2 diabetes, shows CAN was independently associated

with CKD, albuminuria and eGFR in patients with type 2 diabetes. In addition, CAN was an independent predictor of the decline in eGFR over the follow-up period [99].

In a 2014 study by Fleischer J et al, to identify the presence of cardiovascular autonomic neuropathy (CAN) in a cohort of individuals with diabetes in outpatient clinics from 4 different parts of Denmark and to explore the difference between type 1 and type 2 diabetes in relation to CAN, it was found the prevalence of CAN was higher among patients with type 2 diabetes (35%) compared to patients with type 1 diabetes (25%). Multivariate analysis revealed significant associations between CAN and different risk markers in the 2 populations. In type 1 diabetes patients CAN was associated with microalbuminuria ($P < .001$), macroalbuminuria ($P = .011$), simplex retinopathy ($P < .001$), proliferative retinopathy ($P < .001$), and peripheral neuropathy ($P = .041$). Among type 2 diabetes patients CAN was independently associated with high pulse pressure ($P < .01$), BMI ($P = .006$), and smoking ($P = .025$). In this cross-sectional observational study CAN was independently associated with microvascular complication in type 1, whereas in type 2 CAN was associated with macrovascular risk factors [100].

MATERIALS AND METHODS

Source of Data.

The study group included 120 subjects, 60 T2DM patients as cases, who were admitted in R.L Jalappa Hospital attached to Sri Devaraj Urs Medical College, Kolar or attending Diabetic Clinic attached to the hospital and 60 healthy non diabetic volunteers as controls, who were matched for age and gender with the cases.

Informed written consent was obtained from all the patients participating in the study after clearly explaining the study procedure.

The study was approved by the Ethical committee, Sri Devaraj Urs Medical College , Kolar.

Cases were divided into two sub-groups based on the duration of diabetes, i.e, Group 1 with duration of diabetes </= 5yrs and Group 2 duration of diabetes > 5yrs .

Cases were also divided in two sub-groups based on the glycemic control , i.e , Group 1 with HbA1c levels < 7% (T2DM patients with good glycemic control) and Group 2 with HbA1c levels > 7% (T2DM patients with poor glycemic control).

Study Period.

This study was conducted for a period of 1 year from June 2013 to July 2014.

Study Design.

Case – Control Study.

Inclusion Criteria (for cases):

1. T2 DM patients aged > 40yrs who gave informed consent for the study.

Exclusion Criteria (for cases):

1. Patients with cardiac rhythm abnormalities and atrioventricular conduction abnormalities.
2. Patients on drugs known to cause cardiac rhythm abnormalities and atrioventricular conduction abnormalities.
3. Patients with coexisting organic heart disease.
4. Patients who were smokers and alcoholics.

Inclusion Criteria (for controls):

1. Healthy non diabetic individuals aged > 40 years.
2. HbA1c levels less than 6%.

Exclusion Criteria (for controls):

1. Subjects with cardiac rhythm abnormalities and atrioventricular conduction abnormal
2. Subjects on drugs known to cause cardiac rhythm abnormalities and atrioventricular conduction abnormalities.
3. Subjects with coexisting organic heart disease.
4. Subjects who were smokers and alcoholics.

Methods

All the cases and controls who fulfilled the inclusion and exclusion criteria and have given consent to the study were subjected to detailed clinical examination which included bedside tests for cardiovascular autonomic neuropathy. All the cases and controls have undergone the following investigations for evaluation of glycemic control, i.e, FBS, PPBS and HbA1c. Later, the cases were divided into groups based on duration of diabetes and also according to glycemic control. The relationship between CAN and duration of diabetes and glycemic control was evaluated by statistical analysis. The glycosylated Hb level is estimated by cat-ion exchange resin method.

Autonomic Function Tests [61,67].

Tests for sympathetic component.

1) Lying to standing (change in systolic blood pressure).
2) Handgrip test (change in diastolic blood pressure).
3) Cold pressor test (change in diastolic blood pressure).

Tests for parasympathetic component

1) Deep breathing test (delta heart rate).
2) Valsalva maneuver (Valsalva Ratio).
3) Lying to standing (30:15 ratio).

The subjects were instructed not to have coffee, tea cola 12 hours before the tests and were asked to have light breakfast two hours before the tests. The subject was asked to relax in supine position for 30 minutes. The resting heart rate was recorded on a standard ECG from lead two, at a paper speed of 25 mm/sec. BP was measured with sphygmomanometer by the standard auscultatory RivaRocci method. The cardiovascular tests performed are detailed below in the order of execution.

1) **Lying to standing test (LST) – Change in SBP** [61,67]

Blood pressure was recorded in the upper arm by sphygmomanometer in supine position at rest and again recorded 1 minute after standing. The postural fall in systolic blood pressure was taken as a difference between systolic pressure in supine and systolic pressure on 1 min. after standing.

Normal : $\leq$ 10 mmHg. Borderline : 11 – 20 mmHg. Abnormal : $\geq$ 20 mmHg.

2) **Handgrip test (HGT) - Change in DBP** [61,67]

Resting systolic blood pressure was recorded. Subjects were asked to maintain handgrip in other arm at 30 % of maximum voluntary pressure for up to 5 minutes. Systolic pressure was recorded each minute. The difference between the resting systolic pressure and maximum systolic pressure during sustained handgrip was considered for the interpretation.

Normal: $\geq$ 16 mmHg. Borderline: 11 – 15 mmHg. Abnormal: $\leq$ 10 mmHg.

3) Cold pressor test (CPT) - Change in DBP [61,67]

The cold pressor test was evaluated by immersion of subjects left hand (up to wrist) in cold water at 8 ^{0}C for 2 min. in recumbent position. Blood pressure was measured before immersion and 1 min. after immersion of hand. In normal persons immersing of hands in ice water raises the systolic pressure by 15 – 20 mmHg and the diastolic pressure by 10 – 15 mmHg .

Normal: ≥ 16 mmHg. Borderline: 11 – 15 mmHg. Abnormal: ≤ 10 mmHg.

4) Deep breathing test (DBT) - delta heart rate [61,67]

With subject sitting, ECG was recorded in lead II throughout the period of deep breathing. Subject breaths deeply and evenly at 6 breaths per minute (5 sec. in, 5 sec. out) for 3 cycles (30 sec.). The onset of inspiration and expiration were marked on ECG paper. The maximum and minimum R-R interval were measured during expiration and inspiration respectively in each cycle. The heart rate difference during each cycle was measured and average of the 3 differences was considered.

Normal: ≥ 15 beats / min. Borderline: 11 – 14 beats / min. Abnormal: ≤ 10 beats / min.

5) lsalva maneuver (VM) - Valsalva Ratio (VR) [61,67]

The quantitative Valsalva maneuver was performed by blowing with open glottis into a mouthpiece connected to mercury column of a sphygmomanometer. Subjects/patients were asked to maintain 40–50mmHg pressure for 15 sec. The ECG was recorded for 15 sec. during and 30 sec. after the maneuver. Valsalva ratio was calculated by using the following formula.

Valsalva ratio = Longest R-R interval after maneuver.

Shortest R-R interval during maneuver.

Normal: > 1.21. Borderline: 1.11-1.20 . Abnormal: < 1.10.

6) Lying to standing test (30:15 ratio) [61,67]

The ECG limb leads were attached and ECG was recorded in lead II. Subject stands from supine position as quickly as possible. The 30: 15 ratio i.e. ratio of longest R-R interval around 30th beat after standing to shortest R-R interval about 15th beat after standing were considered .

Normal: ≥ 1.04. Borderline: 1.01-1.03. Abnormal: ≤ 1.01.

After the subjects have undergone the above mentioned six autonomic functions tests, the presence of CAN was determined by the following criteria [61,67],

Normal = all test normal or one test borderline.

Early CAN = one test abnormal or two test borderline.

Definite CAN = two tests abnormal.

The relationship between CAN, duration of diabetes and glycemic control was determined .by Statistical Analysis.

CARDIAC AUTONOMIC FUNCTION TESTS [61,67]

TESTS FOR SYMPATHETIC COMPONENT				
		Normal	**Borderline**	**Abnormal**
1	**Lying to Standing Test (LST) - (Change in SBP) in mm Hg**	**<10mm**	**11-20mm**	**>20 mm**
2	**Hand Grip Test (HGT) - (Change in DBP) in mm Hg**	**> 16mm**	**11 – 15mm**	**<10mm**
3	**Cold Pressor Test (CPT) - (Change in DBP) in mm Hg**	**> 16mm**	**11 – 15mm**	**<10mm**
TESTS FOR PARASYMPATHETIC COMPONENT				
		Normal	**Borderline**	**Abnormal**
1	**Deep Breathing Test (DBT) - (Delta Heart Rate)**	**>15bpm**	**11-14bpm**	**<10bpm**
2	**Valsalva Maneuver (Valsalva ratio) – (VR)**	**>1.21**	**1.11 – 1.20**	**<1.10**
3	**Lying to Standing (30: 15 ratio)**	**>1.04**	**1.01 – 1.03**	**<1.01**

Statistical analysis

Data collected was entered into Microsoft excel data sheet and was analyzed using EPI info Version 7 software. Qualitative data was represented in the form of frequencies, proportions and Chi-square test was the test of significance. Quantitative data was represented as Mean, Standard Deviation and Student's t test (Independent t test) and ANOVA (Analysis of Variance) were the tests of significance. p value <0.05 was considered as statistically significant.

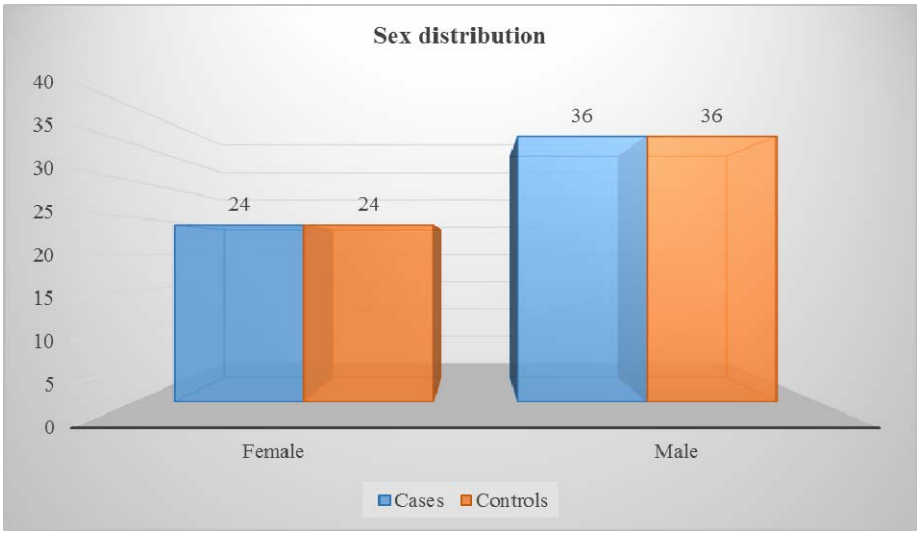
RESULTS

60 cases with diabetes and 60 controls without diabetes were included in the study. Mean age of cases was 52.38 and controls was 51.97. There was no difference in age between two groups because of matching.

Table 1: Sex distribution of the subjects

		Groups		Total
		Cases	Controls	
Sex	Female	24	24	48
	Male	36	36	72
Total		60	60	120

In the study there were equal no of females and males in both cases and controls. There was no difference in sex distribution. This can be attributed to matching of subjects during data collection.



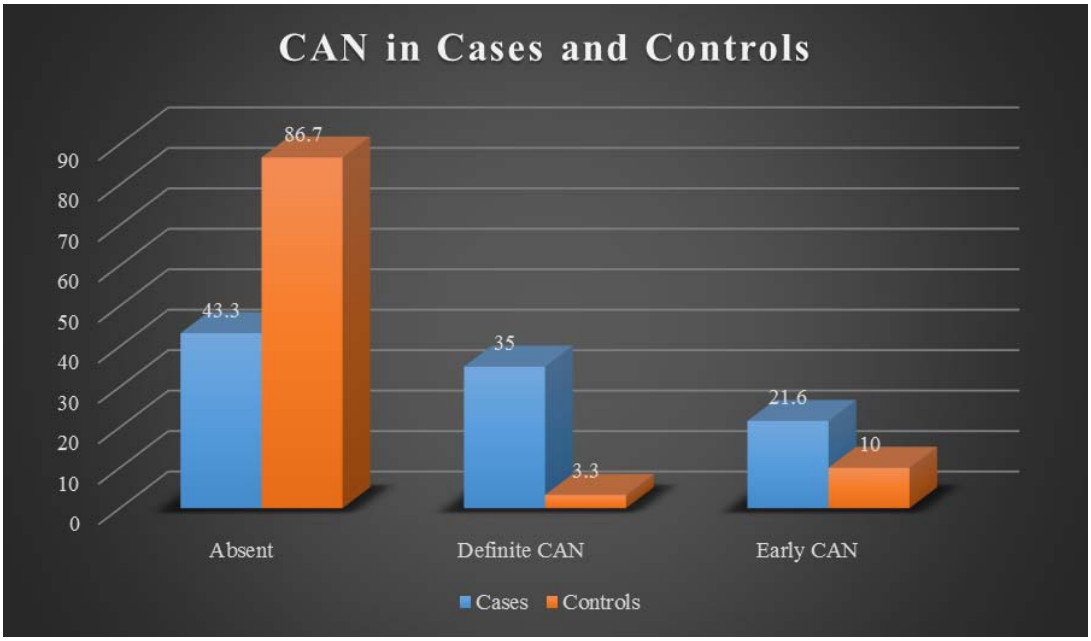
Graph 1: Sex distribution of subjects

Table 2: Cardiac autonomic neuropathy in Cases and Controls

		Groups		Total
		Cases	Controls	
Cardiac autonomic Neuropathy (CAN)	Absent	26	52	78
	Definite CAN	21	2	23
	Early CAN	13	6	19
Total		60	60	120

$\chi 2 = 26.91$, df = 2, p <0.0001**

In the study it was observed that Cardiac autonomic Neuropathy was present in 21 (35%) of diabetic patients, 13 (21.6%) cases had early features of CAN and in 26 (43.3%) cases CAN was absent. Whereas CAN was present in 2 (3.3%) of controls and was absent in 86.6% of controls. This observation was statistically significant i.e. CAN was common in diabetes mellitus.

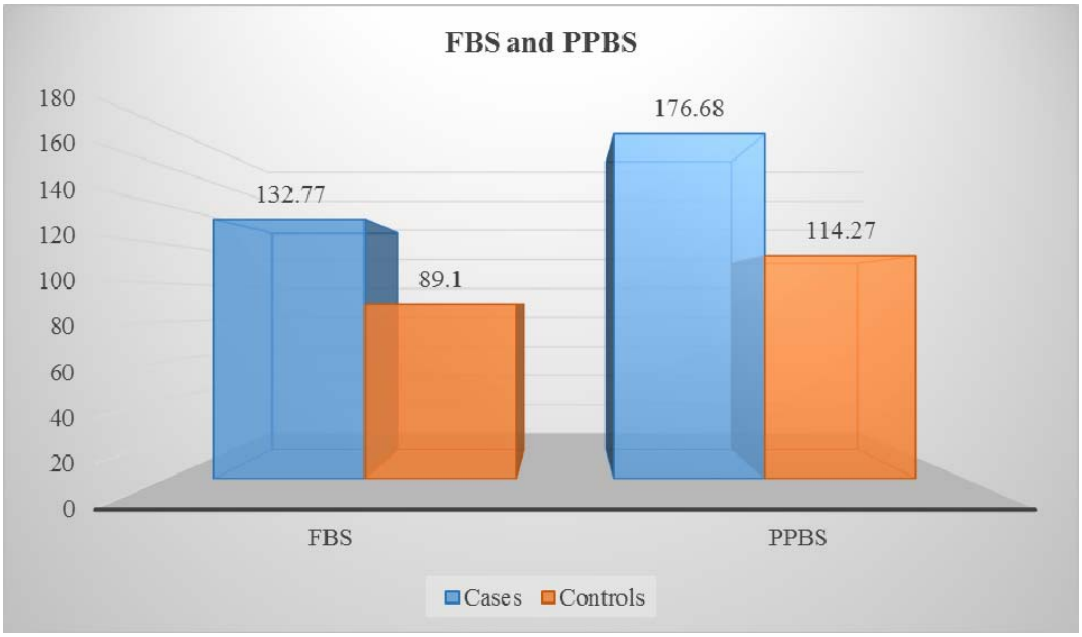


Graph 2: CAN among cases and controls

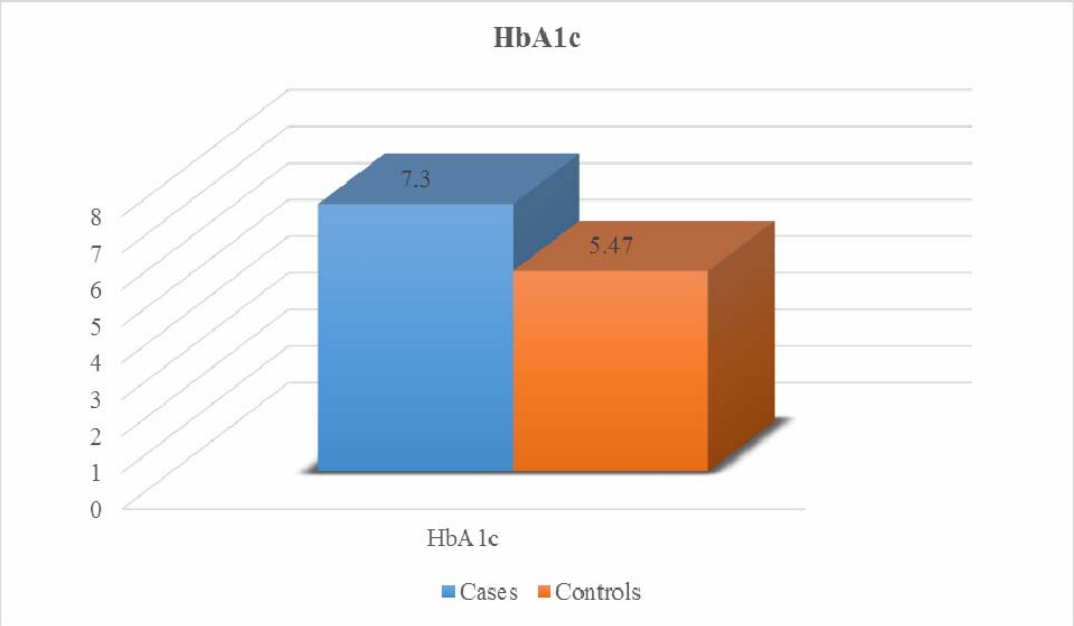
Table 3: Glycemic status among Cases and Controls

		N	Mean	Std. Deviation	t value	p value
FBS	**Cases**	60	132.77	42.399	7.499	<0.0001**
	Controls	60	89.10	15.385		
PPBS	**Cases**	60	176.68	59.595	7.732	<0.0001**
	Controls	60	114.27	18.935		
HbA1c	**Cases**	60	7.30	1.139	11.16	<0.0001**
	Controls	60	5.47	0.566		

It was observed that Mean FBS, PPBS and HbA1c levels were higher in cases than in controls. This difference was statistically significant.



Graph 3: FBS and PPBS levels of Cases and Controls

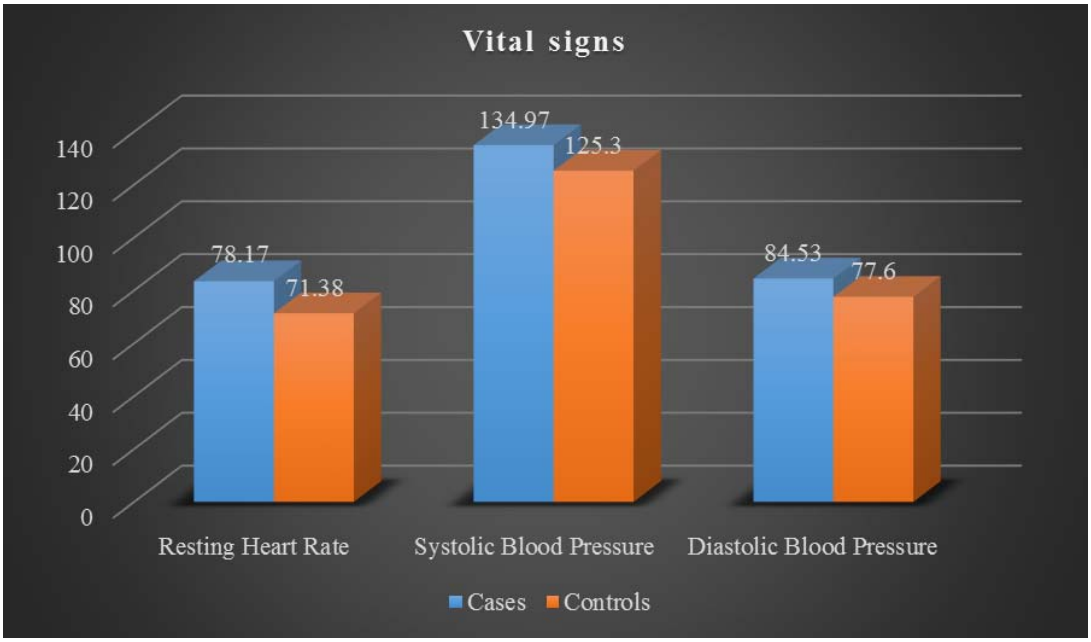


Graph 4: HbA1c levels in cases and controls

Table 4: Vital signs among Cases and Controls

		N	Mean	Std. Deviation	t value	p value
Resting Heart Rate	**Cases**	60	78.17	8.716	3.791	<0.0001**
	Controls	60	71.38	7.381		
Systolic Blood Pressure	**Cases**	60	134.97	9.464	4.21	<0.0001**
	Controls	60	125.30	15.047		
Diastolic Blood Pressure	**Cases**	60	84.53	11.751	3.124	<0.0001**
	Controls	60	77.60	8.057		

In the study it was observed that Mean RHR was more among diabetics, SBP and DBP was high in diabetics compared to non-diabetics. This observation was statistically significant.

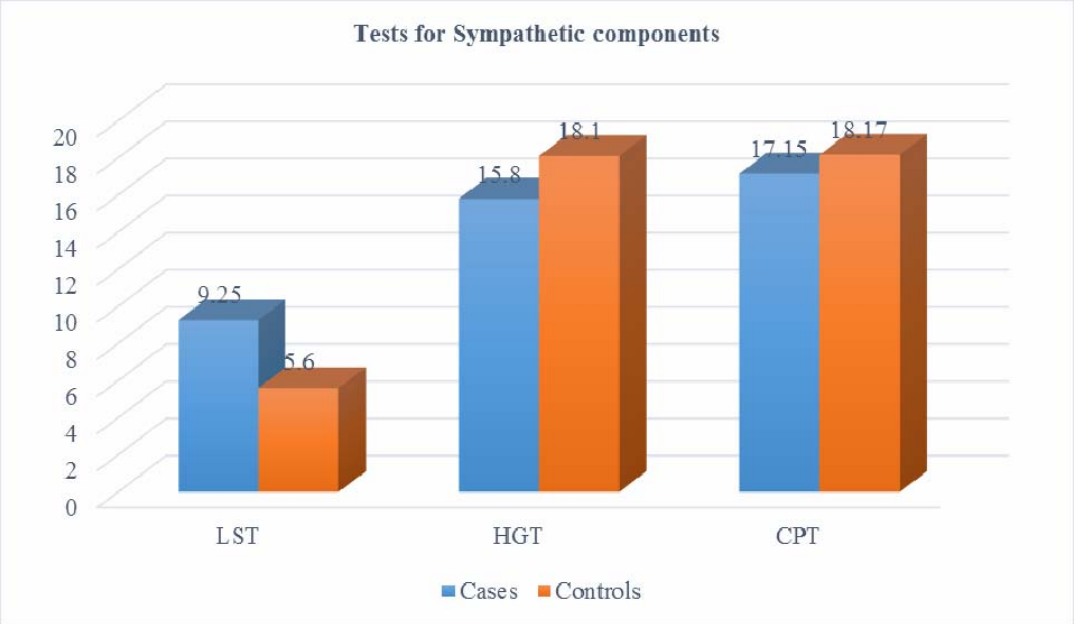


Graph 5: Difference in Vital signs among cases and controls

Table 5: Tests for Sympathetic components among Cases and Controls

		N	Mean	Std. Deviation	t value	p value
Lying to Standing (Change in SBP)in mm Hg	**Cases**	60	9.25	5.513	4.913	<0.0001**
	Controls	60	5.60	1.649		
Hand Grip test (Change in DBP)in mm Hg	**Cases**	60	15.80	3.918	-4.048	<0.0001**
	Controls	60	18.10	2.006		
Cold Pressor test (Change in DBP)in mm Hg	**Cases**	60	17.15	3.502	-1.646	<0.0001**
	Controls	60	18.17	3.258		

It was observed that the mean level of LST was high among cases, HGT and CPT levels was low among cases. This observation was statistically significant.

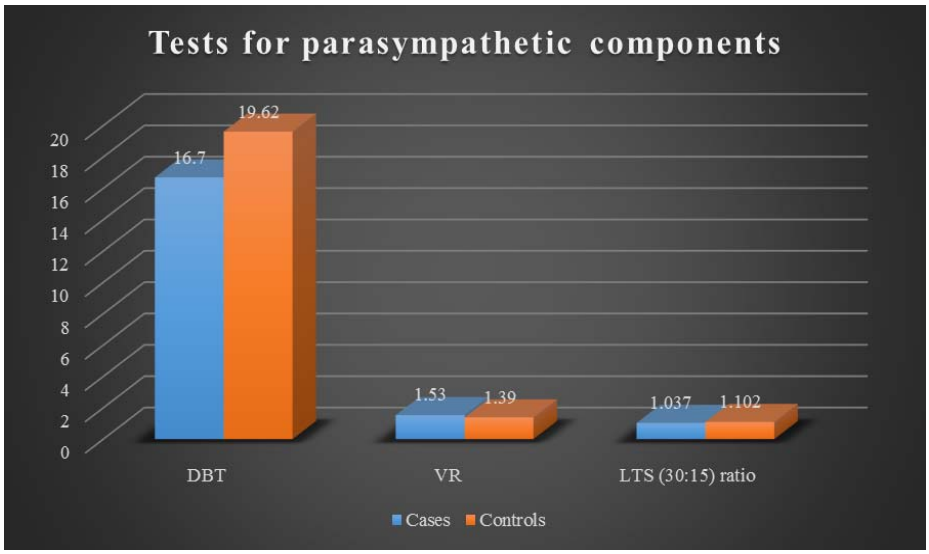


Graph 6: Test results for sympathetic components of autonomic functions tests among cases and controls.

Table 6: Tests for Para Sympathetic components among Cases and Controls

		N	Mean	Std. Deviation	t value	p value
Deep breathing test	**Cases**	60	16.70	5.450	-3.065	0.003**
	Controls	60	19.62	4.961		
Valsalva maneuver ratio	**Cases**	60	1.53	0.454	2.145	0.034*
	Controls	60	1.39	0.197		
Lying to Standing (30:15) Ratio	**Cases**	60	1.037	0.04	-6.73	<0.0001**
	Controls	60	1.102	0.06		

It was observed that DBT and LTS (30:15) ratio was low in cases and VR was high in Cases. This difference between cases and controls was statistically significant.

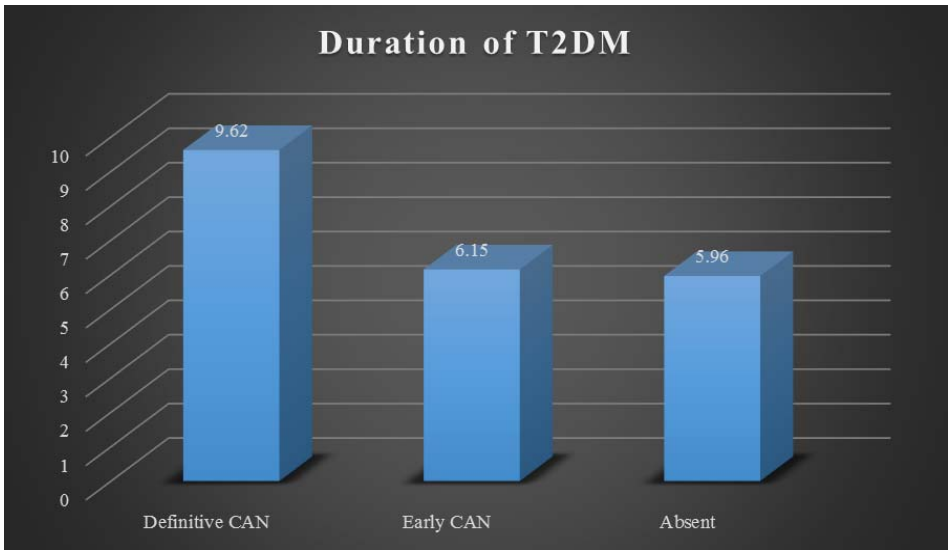


Graph 7: Test results for parasympathetic components of autonomic functions tests among cases and controls.

Table 7: Duration of diabetes in Cardiac autonomic neuropathy

		N	Mean	Std. Deviation	F value	p value
Duration of T2DM	**Definitive CAN**	21	9.62	3.853	8.449	0.001**
	Early CAN	13	6.15	3.184		
	Absent	26	5.96	2.661		
	Total	60	7.28	3.618		

The mean duration of diabetes among cases was 7.28 ± 3.61 years. It was observed that with increase in duration of diabetes there was increase in the risk of Cardiac autonomic neuropathy among diabetics. This observation was found to be statistically significant between the groups and within the groups (F statistic – ANOVA test).

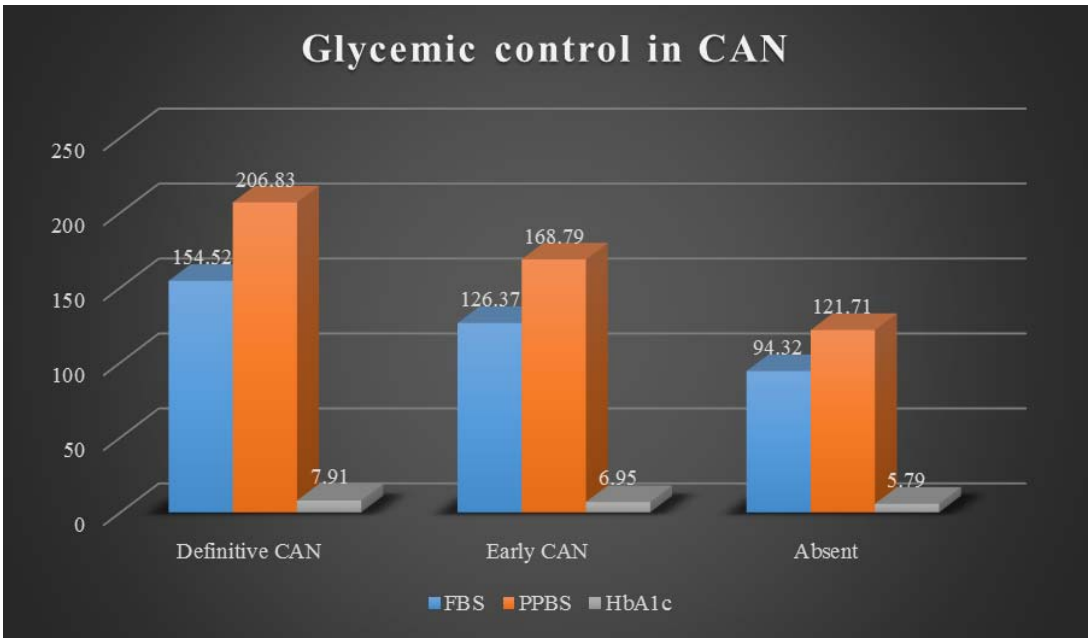


Graph 8: Duration of diabetes in Cardiac autonomic neuropathy

Table 8: Glycemic status in Cardiac autonomic neuropathy

		N	Mean	Std. Deviation	F value	p value
FBS	**Definitive CAN**	23	154.52	46.202	37.966	<0.0001**
	Early CAN	19	126.37	41.990		
	Absent	78	94.32	19.333		
	Total	120	110.93	38.592		
PPBS	**Definitive CAN**	23	206.83	67.198	39.916	<0.0001**
	Early CAN	19	168.79	59.759		
	Absent	78	121.71	23.625		
	Total	120	145.48	54.044		
HbA1c	**Definitive CAN**	23	7.91	1.411	46.416	<0.0001**
	Early CAN	19	6.95	1.268		
	Absent	78	5.79	.691		
	Total	120	6.38	1.285		

It was observed that with higher values of FBS, PPBS and HbA1c there was increased risk of Cardiac autonomic neuropathy. This observation was found to be statistically significant between the groups and within the groups (F statistic – ANOVA test).

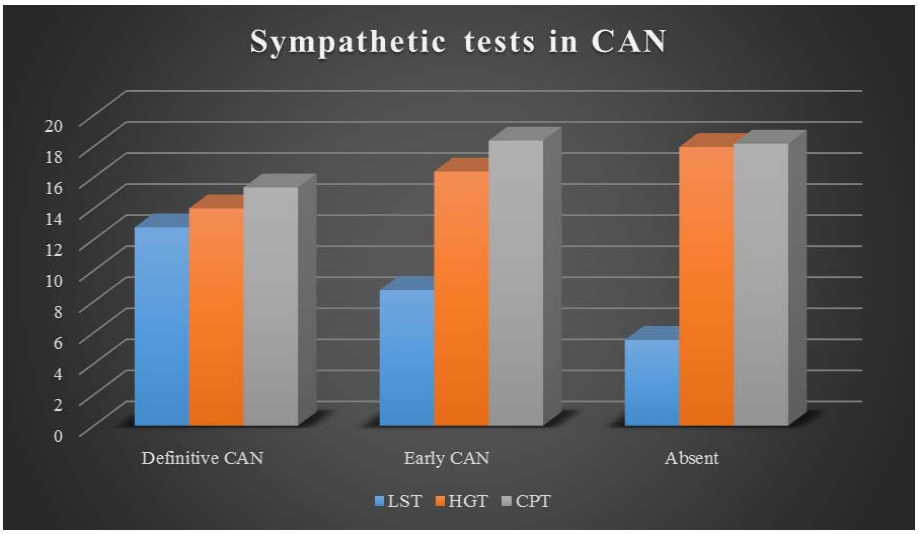


Graph 9: Glycemic status in CAN

Table 9: Sympathetic tests in Cardiac autonomic neuropathy

		N	Mean	Std. Deviation	F value	p value
LST-ΔSBP (in mm Hg)	**Definitive CAN**	23	12.78	6.082	41.330	<0.0001**
	Early CAN	19	8.74	4.544		
	Absent	78	5.53	1.585		
	Total	120	7.43	4.447		
HGT-ΔDBP(in mm Hg)	**Definitive CAN**	23	14.00	4.758	16.502	<0.0001**
	Early CAN	19	16.37	3.483		
	Absent	78	17.96	1.970		
	Total	120	16.95	3.307		
CPT-ΔDBP(in mm Hg)	**Definitive CAN**	23	15.35	5.006	7.263	<0.0001**
	Early CAN	19	18.37	3.218		
	Absent	78	18.17	2.525		
	Total	120	17.66	3.407		

It was observed that among Cardiac autonomic neuropathy subjects there was increase in fall in SBP in LST, decrease in rise in DBP in HGT and CPT. This observation was found to be statistically significant between the groups and within the groups (F statistic – ANOVA test).

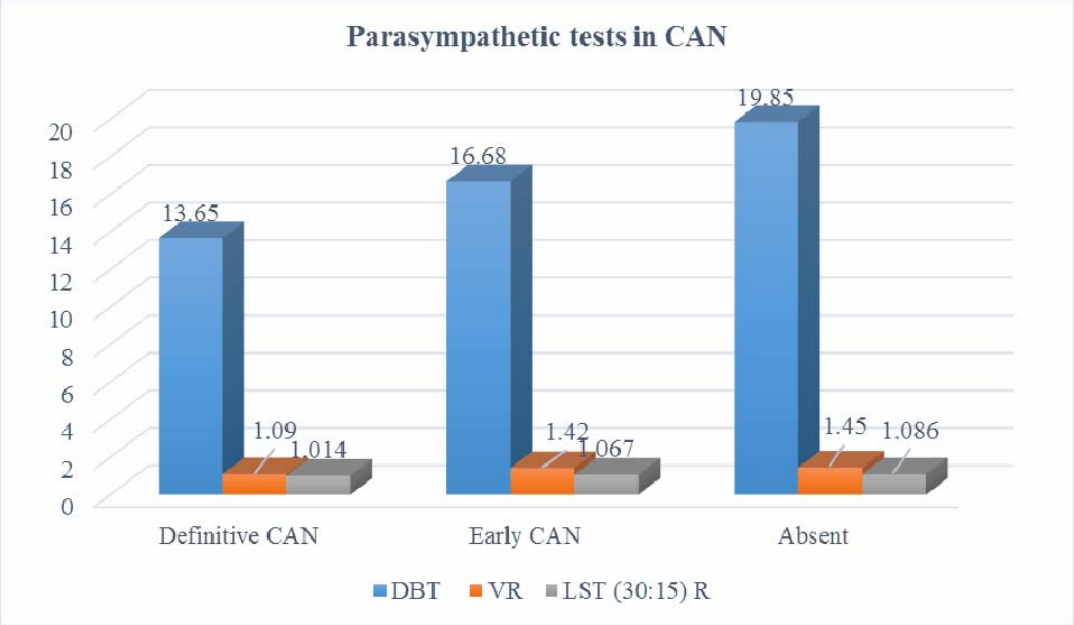


Graph 10 : Sympathetic tests in Cardiac autonomic neuropathy

Table 10: Para sympathetic tests in Cardiac autonomic neuropathy

		N	Mean	Std. Deviation	F value	p value
DBT	**Definitive CAN**	23	13.65	5.749	15.657	<0.0001**
	Early CAN	19	16.68	6.856		
	Absent	78	19.85	3.875		
	Total	120	18.16	5.392		
VR	**Definitive CAN**	23	1.09	0.288	4.112	0.019**
	Early CAN	19	1.42	0.607		
	Absent	78	1.45	0.573		
	Total	120	1.38	0.551		
LST (30:15) Ratio	**Definitive CAN**	23	1.014	0.055	14.397	<0.0001**
	Early CAN	19	1.067	0.051		
	Absent	78	1.086	0.057		
	Total	120	1.069	0.061		

It was observed that among Cardiac autonomic neuropathy subjects there was decrease in DBT VR and LST (30:15) R. This observation was found to be statistically significant between the groups and within the groups (F statistic – ANOVA test).



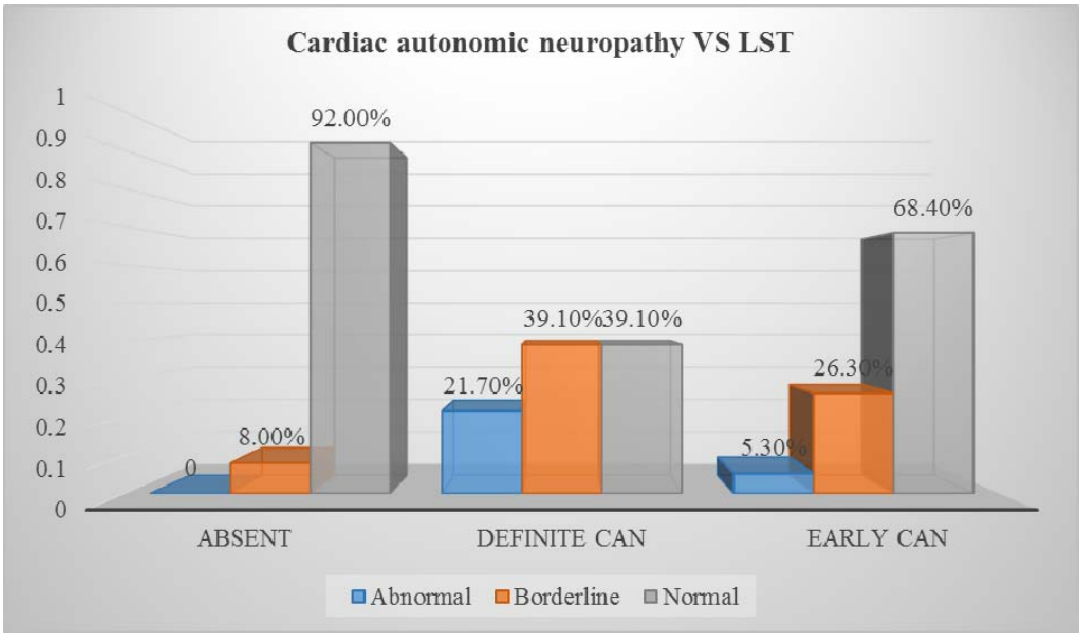
Graph 11: Parasympathetic tests in Cardiac autonomic neuropathy

Table 11: Association between Cardiac autonomic neuropathy and LST

		LST			Total
		Abnormal	Borderline	Normal	
CAN	Absent	0	0	78	78
	Definite CAN	5	9	9	23
	Early CAN	1	5	13	19
Total		6	14	100	120

$\chi 2 = 52.75$, df = 4, p <0.0001**

There was significant association between CAN and abnormal LST.



Graph 12: Association between Cardiac autonomic neuropathy and LST

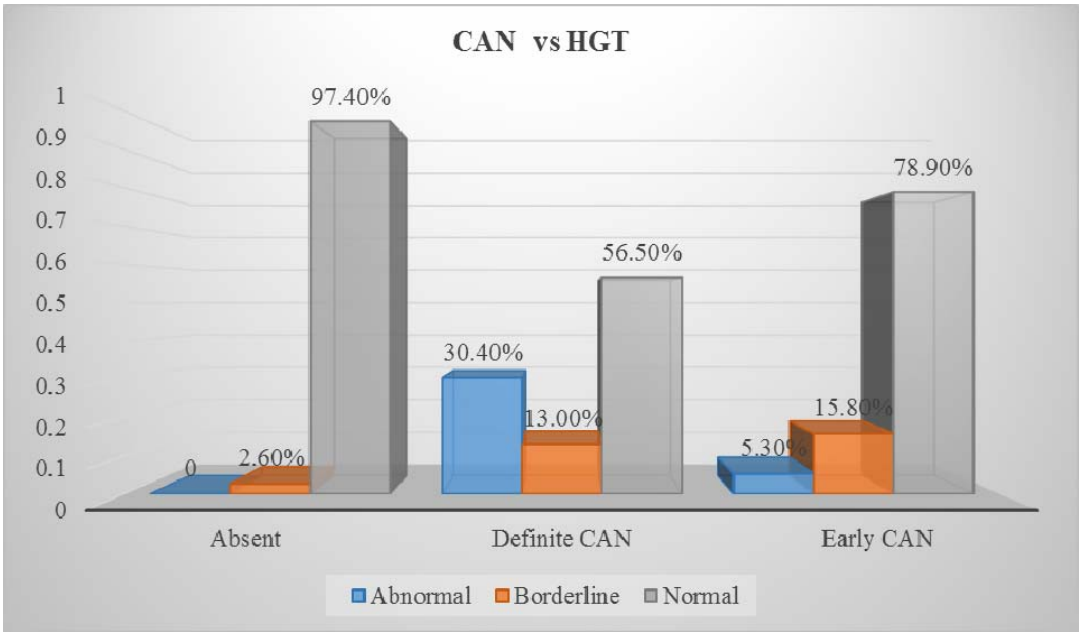
From the Graph it can be inferred that LST was normal in 92% of subjects without CAN and was borderline in 8% of the subjects.

Table 12: Association between Cardiac autonomic neuropathy and HGT

		HGT			Total
		Abnormal	Borderline	Normal	
CAN	Absent	0	2	76	78
	Definite CAN	7	3	13	23
	Early CAN	1	3	15	19
Total		8	8	104	120

$\chi 2 = 34.07$, df = 4, p <0.0001**

There was significant association between CAN and abnormal HGT.



Graph 13: Association between Cardiac autonomic neuropathy and HGT

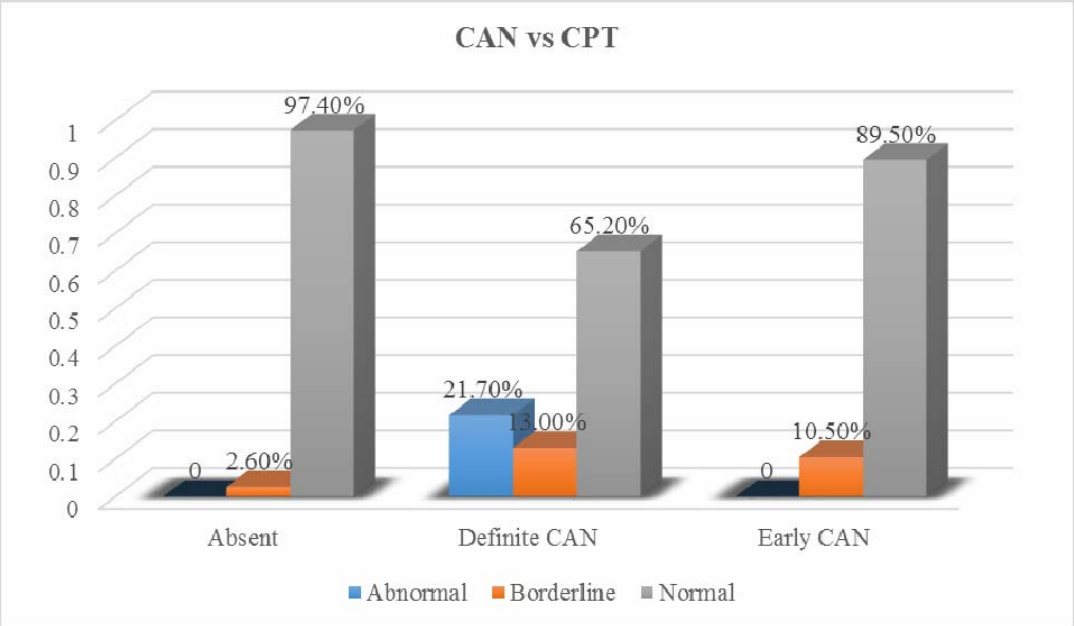
From the Graph in can be inferred that 30% of CAN subjects had abnormal HGT tests and in 97.4% of normal subjects there was no CAN.

Table 13: Association between Cardiac autonomic neuropathy and CPT

		CPT			Total
		Abnormal	Borderline	Normal	
CAN	Absent	0	2	76	78
	Definite CAN	5	3	15	23
	Early CAN	0	2	17	19
Total		5	7	108	120

$\chi 2 = 27.33$, df = 4, p <0.0001**

Among those who had abnormal CPT all the subjects had CAN. There was significant association between CAN and CPT test.



Graph 14: Association between Cardiac autonomic neuropathy and CPT

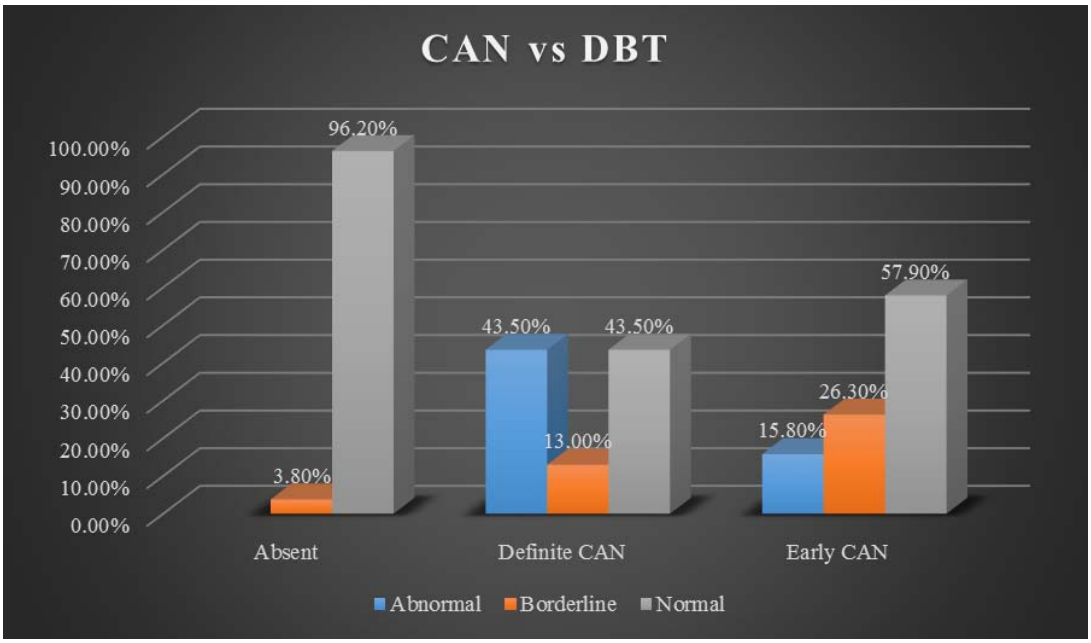
From the Graph it can be inferred that 21.7% of CAN subjects had abnormal CPT and 97.4% of normal CPT test CAN was absent.

Table 14: Association between Cardiac autonomic neuropathy and DBT

		DBT			Total
		Abnormal	Borderline	Normal	
CAN	Absent	0	3	75	78
	Definite CAN	10	3	10	23
	Early CAN	3	5	11	19
Total		13	11	96	120

$\chi 2 = 47.92$, df = 4, p <0.0001**

Among those who had abnormal DBT majority of subjects had CAN. There was significant association between CAN and CPT test.



Graph 15: Association between Cardiac autonomic neuropathy and DBT

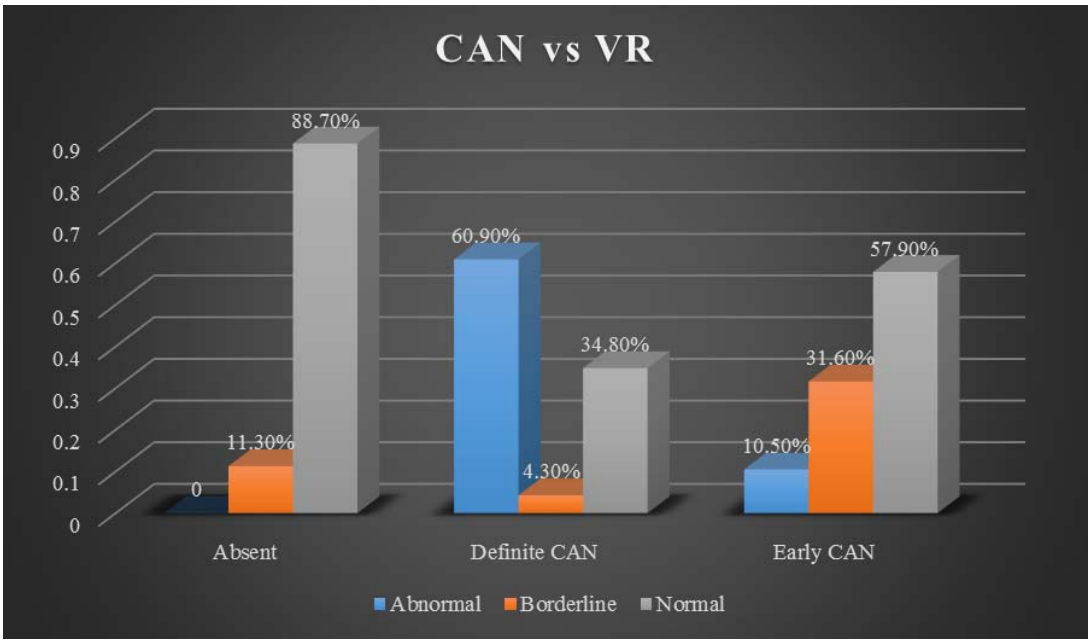
From the Graph it can be inferred that 43.5% of CAN subjects had abnormal DBT and 96.2% of normal DBT test CAN was absent.

Table 15: Association between Cardiac autonomic neuropathy and VR

		VR			Total
		Abnormal	Borderline	Normal	
CAN	Absent	0	1	77	78
	Definite CAN	14	1	8	23
	Early CAN	2	6	11	19
Total		16	8	96	120

$\chi 2 = 81.21$, df = 4, p <0.0001**

Among those who had abnormal VR majority of subjects had CAN. There was significant association between abnormal VR and CAN



Graph 16: Association between Cardiac autonomic neuropathy and VR

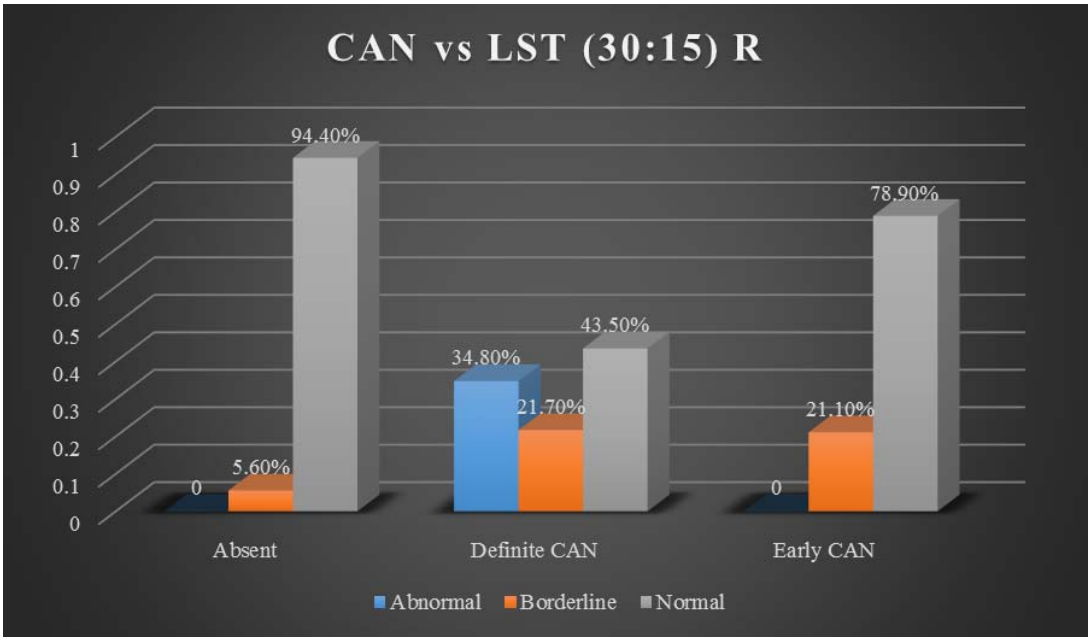
From the Graph it can be inferred that 60.9% of CAN subjects had abnormal VR and 88.7% of normal VR test CAN was absent.

Table 16: Association between Cardiac autonomic neuropathy and LST (30:15) R

		LST (30:15) R			Total
		Abnormal	Borderline	Normal	
CAN	Absent	0	1	77	78
	Definite CAN	8	5	10	23
	Early CAN	0	4	15	19
Total		8	10	102	120

$\chi 2 = 53.51$, df = 4, p <0.0001**

Among those who had abnormal LST (30:15) Ratio all the subjects had CAN. There was significant association between CAN and abnormal LST (30:15) Ratio.



Graph 17: Association between Cardiac autonomic neuropathy and LST (30:15Ratio)

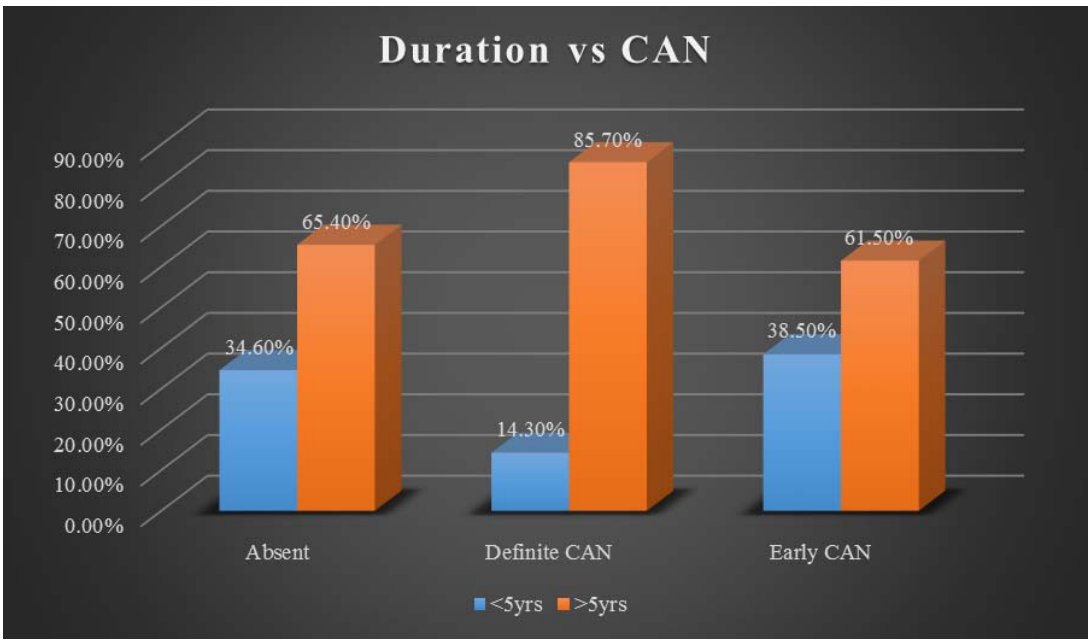
From the Graph it can be inferred that 34.8% of CAN subjects had abnormal LST (30:15) R and 94.4% of normal LST (30:15) R test CAN was absent.

Table 17: Association between Duration and CAN

		Duration		Total
		<5yrs	>5yrs	
CAN	Absent	9	17	26
	Definite CAN	3	18	21
	Early CAN	5	8	13
Total		17	43	60

$\chi 2 = 3.203$, df = 2, p = 0.202

When cases were grouped according to duration of diabetes < 5 years and >5 years, 17.6% and 41.8% of the cases in each group respectively had CAN, which is significant. 85.7% of the cases who had CAN have had diabetes for a duration greater than 5 years.



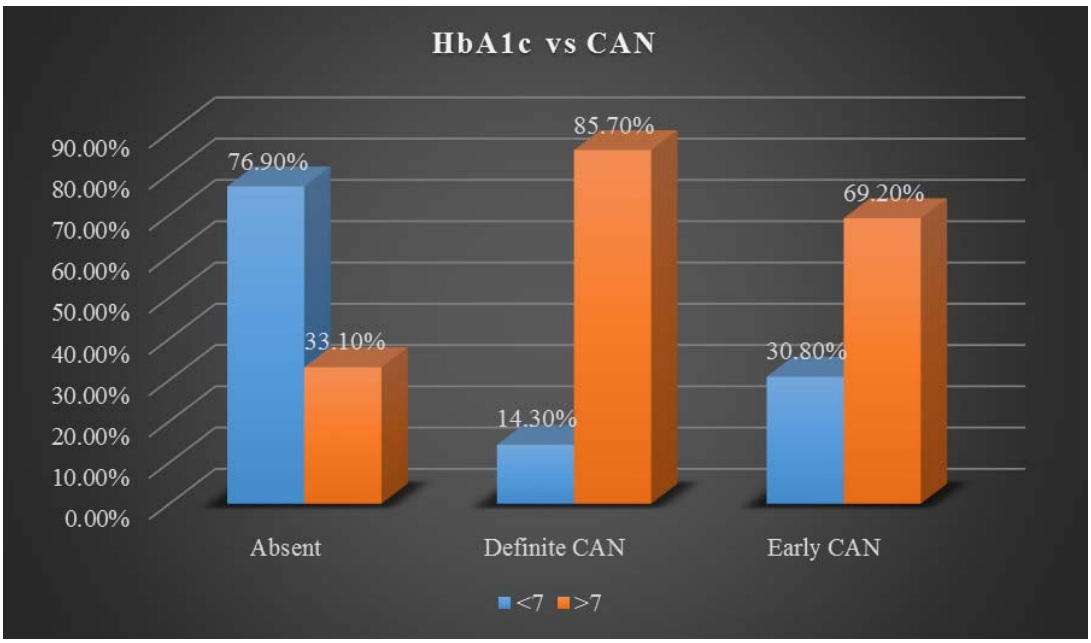
Graph 18: Association between Duration of Diabetes and CAN

Table 18: Association between HbA1c and CAN

		HbA1c		Total
		<7%	>7%	
CAN	Absent	20	6	26
	Definite CAN	3	18	21
	Early CAN	4	9	13
Total		27	33	60

χ 2 = 38.42, df = 2, p = 0.0001**

It was observed that there was significant association between HbA1c and CAN. Among cases with HbA1c >7 54.54% had definitive CAN and 33.3% had Early CAN.

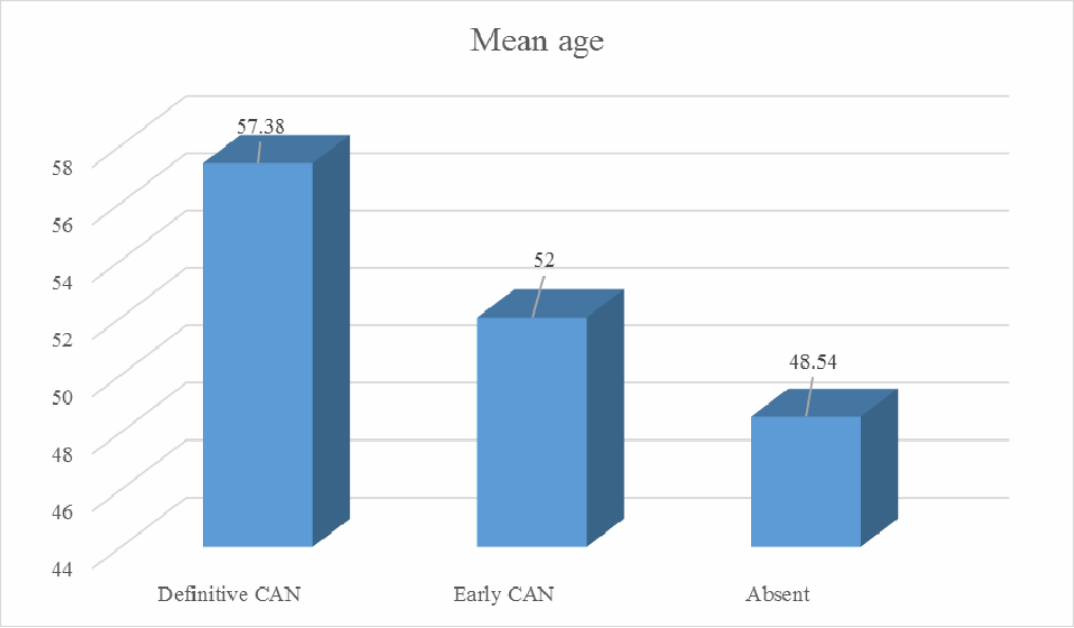


Graph 19: Association between HbA1c and CAN

Table 19: Mean Age in Cardiac autonomic neuropathy

		N	Mean	Std. Deviation	F	p value
Age	**Definitive CAN**	21	57.38	7.290	11.105	0.0001**
	Early CAN	13	52.00	6.178		
	Absent	26	48.54	5.715		
	Total	60	52.38	7.420		

In the study it was observed that Mean age of diabetics without CAN was 48.54 ± 5.7 yrs, Early CAN was 52 ± 6.17 and Definitive CAN was 57.38 ± 7.2 yrs.



Graph 20: Mean Age in Cardiac autonomic neuropathy

DISCUSSION

Diabetic autonomic neuropathy (DAN) is among the least recognized and understood complications of diabetes, despite its significant negative impact on survival and quality of life in people with diabetes. When diabetic neuropathy affects the autonomic nervous system, it can damage the cardiovascular, gastrointestinal, genitourinary and neurovascular systems, and impair metabolic functions such as glucose counter-regulation. Of these, cardiac autonomic neuropathy

(CAN) encompasses damage to the autonomic nerve fibers that innervate the heart and blood vessels, resulting in abnormalities in heart rate control and vascular dynamics. CAN is a significant cause of morbidity and mortality associated with a high risk of cardiac arrhythmias and sudden death. Various studies have shown that the degree of cardiac autonomic dysfunction depend on long term glycemic control, duration of diabetes, age and genetic constitution of the individual. Quantitative autonomic function tests are widely used to assess autonomic function.

This study was undertaken to assess the cardiac autonomic function in patients with T2 DM and to evaluate the relationship between CAN and duration of diabetes, and glycemic control.

In this study, 60 cases (patients with T2DM) and 60 controls (healthy volunteers) have undergone the previously described autonomic function tests, investigations for glycemic control and the data obtained was statistically evaluated.

In this study, the mean age of cases was 52.38 ± 7.42 years and controls was 51.97 years. There was no significant difference in age between two groups because of matching. In the study it was observed that mean age of diabetics without CAN was 48.54 ± 5.7 yrs, Early CAN was 52 ± 6.17 and Definitive CAN was 57.38 ± 7.2 yrs. There was a statistically significant relationship (p value - 0.0001) between CAN and increasing age among the diabetics. There were 24 females and 36 males in both cases and controls. There was no significant difference in sex distribution. This can be attributed to matching of subjects during data collection. The age and sex distribution of the cases has been shown in Table 17 and Tab 1 respectively.

In this study it was observed that Cardiac Autonomic Neuropathy was present in 21 (35%) of the diabetic patients, 13 (21.6%) cases had early features of CAN and in 26 (43.3%) cases CAN was absent. Whereas CAN was present in 2 (3.3%) of controls and was absent in 86.6% of controls. This observation was statistically significant i.e. CAN was common in diabetes mellitus. The incidence of Definite CAN among patients with T2DM in this study is 35% and it was 56.6% when patients with early CAN was also included. The incidence of autonomic neuropathy in diabetics, ranged from 17 to 68% in other studies. However, Aaron. I. Vinik, Raelene E. Maser, et al[101] in their study state that cardiovascular autonomic neuropathy incidence in diabetics varies from 7.7% (Type 1) to even 90% (in pancreatic transplants). J.M.Pappachan, J.Sebastian, et al[102], in their study, showed a prevalence of cardiovascular autonomic neuropathy in 60% of the 100 cases of diabetics studied. A.Goel, Ruchika Agarwal, et al [103], 2004 have reported 29 out of 75 diabetic patients (39%) to have dysautonomia. Similar results were seen with Oluranti B.familoni, Olatunde odusan, Taiwo.H.Raimi[104], have showed a prevalence of 37% of

dysautonomia among the diabetics under study. M.Lakhotia, P.K.D. Shah[105], et al., in their study, showed that 8 out of 12 Type 1 and 24 out of 38 Type 2 diabetics had dysautonomia. Noronha J.L., Bhandarkar S.D[106]., et al., in their study, found that 78.8% of the diabetics had autonomic neuropathy, of which more than 76%, of the total study, had more than one abnormal test present. Masoaka[107] et al. in a study of 105 diabetics, both Type 1 and Type 2, found an incidence of 50%.

The mean duration of diabetes in cases was 7.28 $\pm$ 3.61 years. In cases with definite CAN the mean duration was 9.62 $\pm$ 3.85 years and in cases with Early CAN, the mean duration of diabetes was 6.15 $\pm$ 3.18 years and in diabetics without CAN, it was 5.96 $\pm$ 2.66 years. It was observed that with increase in duration of diabetes there was increase in the risk of Cardiac autonomic neuropathy among diabetics. This observation was found to be statistically significant between the groups and within the groups (p value = 0.001) (F statistic – ANOVA test). This has been illustrated in Table 7 and Graph 8. This finding was in agreement with many of the previous studies which were conducted for finding the prevalence of CAN. Mohan et al [108] studied the prevalence of CAN in 336 patients with T2DM2 in south India. There was an increase in prevalence of CAN with duration of diabetes. In 0-5 years group the prevalence of autonomic dysfunction was 28.2%. In this study, the prevalence of autonomic dysfunction in 0-5yrs group was 17.6% and it was 41.8 % in cases with duration of diabetes greater than 5 years. Toyry J P et al[109] studied the clinical significance of autonomic neuropathy in T2DM. A total of 133 patients with newly diagnosed T2 DM(70 men) and 144 control subjects (62 men) were examined at baseline and after 5 and 10 years of follow-up. The frequency of autonomic dysfunction at baseline, at 5 yrs and 10yrs was 4.9%, 19.6% and 65% respectively.

In the study it was observed that Mean RHR was more among diabetics, SBP and DBP was high in diabetics compared to non-diabetics. This observation was statistically significant. Resting tachycardia and a fixed heart rate are characteristic late findings in diabetic patients with vagal impairment. Resting heart rates of 90 to 100 bpm and occasional heart rate increments up to 130 bpm occur. The highest resting heart rates have been found in patients with parasympathetic damage, occurring earlier in the course of CAN than sympathetic nerve function; in those with evidence for combined vagal and sympathetic involvement, the rate returns toward normal but remains elevated [26].

In Lying to Standing Test, to evaluate the sympathetic component of Autonomic nervous system, the mean fall in SBP in subjects with Definite CAN was 12.78 $\pm$ 6.02 mm Hg, which when compared to 5.53 $\pm$ 1.58 mm Hg in subjects without CAN, was statistically significant(p value - <0.0001). Blood pressure response to standing was significantly reduced in diabetics. Decrease in SBP on standing from lying was highly significant between controls and diabetics with poor control, also between well controlled and poorly controlled diabetics ($P<0.001$).

Ewing DJ, Martyn CN, Young RJ and Clarke BF assessed autonomic function in 774 diabetic subjects, using all 5 simple, non-invasive cardiovascular autonomic function tests for 10 years. And observed abnormal blood pressure variation from lying to standing in 20% of the subjects [60]. Kempler P, Tesfaye S, Chaturvedi N, Stevens L. K., Webb D.J, Eaton S et al. have concluded that a fall of more than 20 mmHg in SBP after standing up seemed to be most reliable criterion for the assessment of orthostatic hypotension in the diagnosis of autonomic neuropathy in

patients with Type– I diabetes mellitus in a study involving 3007 randomly selected Type –I diabetic patients on blood pressure response to standing in diagnosis of autonomic neuropathy: The EURODIAB IDDM complications study [114].

The mean increase in the DBP in Hand Grip Test was 15.8 ± 3.91mm Hg in Cases which was low when compared to 18.1 ± 2.00 mm Hg in controls. This finding was statistically significant when diabetic patients were compared to healthy volunteers. Blood pressure response to sustained handgrip was significantly reduced in diabetics. Increase in DBP to sustained handgrip was highly significant between controls and well controlled diabetics, controls and poorly controlled diabetics also between well controlled and poorly controlled diabetics.

In 1978, a study on "Cardiovascular reflexes and Autonomic neuropathy" by Ewing. D. J. described an abnormally small blood pressure increase during sustained handgrip has been found in unselected diabetic subjects, in diabetic subjects with autonomic neuropathy and also in patients with chronic renal failure [115]. Popovic et al studied effect of sustained hand grip on BP variation in 90 subjects further divided in to groups of 30 each as Diabetic type I Diabetic type II and non -diabetic control. They observed an abnormal BP variation in type II Diabetics than compared to other groups [116].

The mean increase in the DBP in Cold Pressor Test was 17.15 ± 3.50 in Cases which was lower when compared to 18.17 ± 3.25 mm Hg in Controls and was statistically significant. Hines Edgar A and Brown George E in 1936, in their study on "Cold pressor test for measuring reactibility of the blood pressure.Data concerning

571 normal and hypertensive subject". The normal reactors (minimal or normal reaction of blood pressure to cold test), normal hyper reactors (subjects with normal level of blood pressure who react excessively to cold test) and hypertensive subjects have found the mean increase in normal reactors was 11.4 mmHg systolic and 10.6 mmHg diastolic pressure. The normal hyper reactors showed mean rise in systolic blood pressure of 29.4 mmHg and 24.5 mmHg diastolic blood pressure. The values for the mean rise in systolic blood pressure in hypertensives were 47.2 mmHg and 34.3 mmHg diastolic pressure [117].

In study done by Sayinal P.S, Sozen T, Ozdogan M entitled "Cold pressor test in diabetic autonomic neuropathy" in 1994, the CPT was applied to a group of diabetic patients (n=33) and control group (n=15), the mean systolic cold pressor response in diabetic patients was found similar to controls (9 ± 1.4 vs 10.6 ± 1.2 mmHg). However the mean diastolic cold pressor response was significantly lower in diabetic patients as compared with the control group (7.7 ± 1.0 Vs 12.0 ± 1.1mmHg $p < 0.05$). In patients with autonomic neuropathy the diastolic cold pressor response was smaller than control (6.9 ± 1.2 vs 12.0 ± 1.1 mmHg $p<0.05$), however in patients without autonomic neuropathy it was not significantly different from controls [118].

In this study, about 43.5% of the subjects who had CAN, had an abnormal Deep Breathing Test and in 96.2% of the subjects having normal DBT, CAN was absent. This was statistically significant. DBT was significantly decreased ($p<0.0001$) in both the diabetic groups when compared to controls. Further there was a significant difference ($p<0.0001$) between the well-controlled and poor controlled diabetic groups. This shows that there is progressive parasympathetic dysfunction in diabetics

but it is more in poorly controlled diabetics. The basis of this finding is multifactorial and may be at multiple levels of neuraxis including peripheral and central mechanism as reviewed earlier under effect of glycemic control over cardiac autonomic dysfunction. HR response to breathing is a normal phenomenon and is due primarily to fluctuations in parasympathetic output to heart. During inspiration impulses in vagi from stretch receptors in lungs inhibit the cardio-inhibitory area in medulla oblongata. The tonic vagal discharge that keeps the heart rate slow decreases as a result, the heart rate rises. In diabetes, loss of vagal tone (vagal denervation) is responsible for reduced heart rate response to deep breathing in diabetes. Parasympathetic fibers being the longest fibers are affected first due to atherosclerotic changes of vasa nervosum. In diabetes, there is a cluster of metabolic and hemodynamic abnormalities, including a disadvantageous lipid profile and altered diurnal blood pressure rhythm. The finding of the present study is in conformity with earlier studies.

Oikawa N et al, studied Heart rate (HR) variations during deep breathing in 162 healthy subjects and 168 diabetics by use of an instantaneous-HR -change continuous recorder. As indices of HR variations, the mean of HR during deep breathing and the standard deviation (SD) of the HR were determined. . The 95% confidence limits were calculated for the normal range and the values below normal range were defined as abnormal. In healthy subjects, the values for each test declined with age and the log-transformed data fitted the linear regression. In diabetics, the incidence of abnormal response were 38%, The Deep breathing difference was found to be the most sensitive index for the autonomic neuropathy [119].

Sundkvist G, Almer L-O, Lilja B. in 1979 in the study entitled "Respiratory influence on heart rate in diabetes mellitus" have observed a low Expiratory: Inspiratory (E:I) ratio in two people in each group of control (n = 25) and diabetic without sensory neuropathy (n = 23). There was no difference in the mean values between these two groups. Ten of eighteen with sensory neuropathy had abnormal E: I ratio and the mean values for diabetic with sensory neuropathy was significant when compared to control and diabetic without sensory neuropathy [120].

In this study, 60.9% of the subjects with CAN, had abnormal valsalva ratio and in 88.7% of the subjects having normal VR, CAN was absent. Bhatia S.G, Sainani S.G, Nayak N.J and Diwate P.G. in 1976 in a study entitled "Valsalva manoeuver as a test of autonomic neuropathy in diabetes mellitus" on 35 healthy control and 100 randomly selected diabetic patients in which 26 had autonomic neuropathy and 74 without autonomic neuropathy. The response with ratio below 1.2 was considered to be an abnormal. The occurrence of abnormal Valsalva response in patients with autonomic neuropathy was found in 21 patients out of 26 and was statistically significant. 5 diabetic with autonomic neuropathy have shown normal response. Three patients without autonomic neuropathy showed abnormal valsalva response and 71patients showed normal response [121]. The heart rate response to Valsalva maneuver relies to some extent on the integrity of sympathetic as well as parasympathetic pathways. Parasympathetic fibers being the longest fibers are affected first due to atherosclerotic changes of vasa nervosum. In diabetes, changes in metabolic and hemodynamic abnormalities, including a disadvantageous lipid profile, altered diurnal blood pressure rhythm. The probable etiology is explained under

review of literature. The finding of the present study is in conformity with earlier studies.

About 34.8% of CAN subjects had abnormal LST (30:15) Ratio and 94.4% of normal LST (30:15) R test CAN was absent. Spallone V et al performed cardiovascular autonomic function test in 35 normotensive diabetic subjects by measuring HRV from lying to standing, using 24 hour ECG recording and found significant variation in HRV from lying to standing ($P < 0.02$) [122]. Smith SA performed VR in 59 diabetic patients and found that 35 had normal, 6 had borderline and 18 had abnormal results compared to other autonomic function tests VR correlated maximally with sinus arrhythmia tests, repeat measurements of ratios in healthy and diabetic subjects yielded coefficient of variation of 15.4% and 10.5% respectively [123].

Among the tests for evaluating the parasympathetic component of Autonomic Nervous system, Deep Breathing test and Lying to Standing Test were statistically significant in cases when compared to controls, whereas Valsalva maneuver was not statistically significant. Vinik et al[109] found that parasympathetic damage occurs earlier than sympathetic damage in diabetic cardiovascular autonomic neuropathy which also is comparable to our study, as the Deep Breathing Test was abnormal in most of the cases with CAN when compared with other autonomic function tests.

In 85.71 % of the cases with Definite CAN, HbA1c levels were greater than 7% (Poorly Controlled T2 DM) when compared to 14.29 % who had HbA1c levels less than 7%, which was significant. Among the 13 cases with Early CAN 69.2% of the cases had poorly controlled T2DM. It was observed that there was significant association between HbA1c and CAN. Among cases with HbA1c >7%, 54.54% had

definitive CAN and 33.3% had Early CAN. HbA1c is an index of long-term blood glucose concentrations [110].

Sustained hyperglycemia levels leads to vascular and metabolic complications resulting in severe autonomic dysfunction. In the poorly controlled diabetics (HbA1c > 7%) long-standing hyperglycemia induced pathological changes intrinsic to neurons [111] may be the probable cause for the noticed autonomic dysfunction in our study. J.M.Pappachan, J.Sebastian, et al [102], in their study, also showed that incidence of diabetic autonomic neuropathy increased with increasing duration and poor glycemic control. Early observations by researchers that near normal glycemic control seems to be the most effective way to delay the onset of CAN and arrest its progression. Hence it is important in emphasizing tight glycemic control for individuals with autonomic dysfunction with re-education of the patient with regard to need for regular monitoring and hypoglycemia.

Thus, timely identification of autonomic dysfunction in diabetic patients may expedite end organ prophylaxis such as the use of ACE inhibitors and aspirin and the use of pharmacological and non-pharmacological interventions to improve blood pressure and lipid control. Improved nutrition and reduced alcohol and tobacco consumption are additional options available to patients with diabetes who are identified with autonomic nerve dysfunction [112]. Early identification of CAN permits timely initiation of therapy with the antioxidant alpha-lipoic acid and Vitamin E, which appears to slow or reverse progression of neuropathies in some studies [113].

CONCLUSIONS

The following conclusions are drawn from this study:

1) Cardiac Autonomic Neuropathy is common in Diabetics compared to healthy individuals.
2) Cardiac Autonomic Neuropathy is associated with increase in the age of the patient and duration of diabetes.
3) Cardiac autonomic neuropathy is associated with poor glycemic control.

SUMMARY

The study was conducted in R.L Jalappa Hospital attached to Sri Devaraj Urs Medical College, Tamaka, Kolar. 60 cases (Patients with T2 DM) and 60 controls (healthy volunteers) who were matched for age and gender, have undergone standard Cardiac Autonomic Function tests, and investigated for glycemic control with FBS, PPBS and HbA1c levels. The objective of the study was to assess the cardiac autonomic function in patients with Type2 Diabetes Mellitus (T2 DM) for detection of CAN and also to evaluate the relationship between CAN and duration of diabetes, and glycemic control in T2 DM.

A total of 60 cases (T2 DM patients) and 60 controls (healthy volunteers) have participated in the study.

- Case-Control Study.
- Mean age of cases was 52.38 years and controls was 51.97years.
- 24 females and 36 males among both cases and controls.
- No significant difference in mean age and gender distribution among cases and controls due to matching of cases and controls.
- Cardiac autonomic Neuropathy was present in 21 (35%) of diabetic patients, 13 (21.6%) cases had early features of CAN.
- Mean age of diabetics without CAN was 48.54 ± 5.7 years, Early CAN was 52 ± 6.17 yrs and Definitive CAN was 57.38 ± 7.2 yrs.

- Mean duration of diabetes among cases was 7.28 ± 3.61 years.
- 85.7% of the cases who had CAN have had diabetes for a duration greater than 5 years.
- Cardiac Autonomic Neuropathy is associated with increase in the age of the patient and duration of diabetes.
- Among cases with HbA1c >7%, 54.54% had definitive CAN and 33.3% had Early CAN.
- It was observed that with higher values of FBS, PPBS and HbA1c there was increased risk of Cardiac autonomic neuropathy.
- Hence we can infer that CAN is common in Diabetics compared to healthy individuals, and is associated with increase in the age of the patient and duration of diabetes and poor glycemic control.

BIBLIOGRAPHY

1. WHO data. Available from www.who.int/diabetes . Accessed August 2014.
2. Jayaram BM et al: Type 2 Diabetes Mellitus and its complications: A preventive program,book 2008.
3. Anjana RM, Pradeepa R, Deepa M, et al. ICMR-INDIAB Collaborative Study Group: Prevalence of diabetes and prediabetes in rural and urban India: Phase 1 results of the Indian Council of Medical Research-INdiaDIABetes (INDIAB) study. Diabetologia 2011; 54:3022-3027.
4. Maser RE, Lenhard MJ, DeCherney GS. Cardiovascular autonomic neuropathy: the clinical significance of its determination. Endocrinologist 2000; 10: 27–33.
5. Schumer MP, Joyner SA, Pfeifer MA. Cardiovascular autonomic neuropathy testing in patients with diabetes. Diabetes Spectrum 1998; 11: 227–231.
6. Rodica Pop-Busui .Cardiac Autonomic Neuropathy in Diabetes. Diabetes Care 2010; 33: 434-441.
7. Pfeifer MA, Weinberg CR, Cook DL, Reenan A, Halter JB, Ensinck JW, et al. Autonomic neural dysfunction in recently diagnosed diabetic subjects. Diabetes Care 1984; 7: 447–453.
8. Low PA. Laborotory Evaluation of Autonomic Function. In: Low PA, editor. Clinical Autonomic Disorders. 2nd ed. New York: Lippincott-Raven Publishers; 1997; 179–208.
9. Khoharo HK, Ansari S, Ali Shaikh I, Qureshi F. J Coll Physicians Surg Pak. 2009; 19(4):232-235.
10. Tortora G.J and Grabowsik S. R. Principles of anatomy and Physiology. 6th Edn. Harper Collins college publishers; 1996. 572p.

11. Ganong W. F. Review of Medical Physiology. 19th edn. NJ: Printice- Hall International Inc, 1997. 560-566pp.
12. Best and Taylor's. Physiological Basis of Medical Practice. 11th edn. John B. West ed, Baltimore, Lundon, Williams and Winkins; 1985. 271- 272 pp.
13. Ansari M, Alexander M, Tutar A, Massie BM. Incident cases of heart failure in a community cohort: importance and outcomes of patients with preserved systolic function. Am Heart J. 2003; 146: 115–120.
14. Purves D, Augustine GJ, Fitzpatrick D, et al. Autonomic Regulation of Cardiovascular Function. Neuroscience. 2nd edition. Sunderland (MA): Sinauer Associates; 2001.
15. Maser and Lenhard. Cardiovascular Autonomic Neuropathy in Diabetes. J Clin Endocrinol Metab 2005; 90(10):5896–5903.
16. Edwards JL, Vincent AM, Cheng HT, Feldman EL. Diabetic neuropathy: mechanisms to management. Pharmacol Ther 2008; 120:1–34.
17. Bell JI, Hockaday TDR. Diabetes mellitus. Oxford Text book of Medicine. 3rd Edition.Vol 2:1996:1448-1504.
18. Vinik AI, Erbas T. Recognizing and treating diabetic autonomic neuropathy. Cleve Clin J Med 2001; 68:928–944.
19. Ziegler D: Diabetic cardiovascular autonomic neuropathy: prognosis, diagnosis and treatment. Diabetes Metab Rev 1994; 10:339–383.
20. Diabetes Control and Complications Trial Research Group. The effect of intensive diabetes therapy on measures of autonomic nervous system function in the Diabetes Control and Complications Trial (DCCT). Diabetologia 1998; 41: 416–423.

21. Kennedy WR, Navarro X, Sutherland DE. Neuropathy profile of diabetic patients in a pancreas transplantation program. Neurology 1995; 45: 773–780.
22. Ziegler D, Gries FA, Muhlen H, et al. Prevalence and clinical correlates of cardiovascular autonomic and peripheral diabetic neuropathy in patients attending diabetes centers. The Diacan Multicenter Study Group. Diabete Metab 1993; 19: 143–151.
23. Spallone V, Ziegler D, Freeman R, et al. Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management. Diabetes Metab Res Rev 2011; 27: 639–653.
24. Singh JP, Larson MG, O'Donnell CJ, et al. Association of hyperglycemia with reduced heart rate variability (The Framingham Heart Study). Am J Cardiol 2000; 86: 309–312.
25. Aaron I Vinik et al. Diabetic Cardiac Autonomic Neuropathy ,Inflammation and Cardiovascular Disease. J Diabetes Invest 2013; 56: 435 – 445.
26. Hage FG, Iskandrian AE. Cardiovascular imaging in diabetes mellitus. J Nucl Cardiol 2011; 18: 959–965.
27. Vinik AI, Ziegler D. Diabetic cardiovascular autonomicneuropathy. Circulation 2007; 115: 387–397.
28. Auton J. The definition of orthostatic hypotension, pure autonomic failure, and multiple system atrophy. Nerv Syst 1996; 58: 123–124.
29. Low PA, Walsh JC, Huang CY, et al. The sympathetic nervous system in diabetic neuropathy. A clinical and pathological study. Brain 1975; 98: 341–356.

30. 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. 1989; 70:591–597.
31. Kitamura A, Hoshino T, Kon T, Ogawa R. Patients with diabetic neuropathy are at risk of a greater intraoperative reduction in core temperature. Anesthesiology. 2000; 92:1311–1318.
32. Sobotka PA, Liss HP, Vinik AI. Impaired hypoxic ventilatory drive in diabetic patients with autonomic neuropathy. J Clin Endocrinol Metab.1986; 62:658–663.
33. Ambepityia G, Kopelman PG, Ingram D, Swash M, Mills PG, Timmis AD. Exertional myocardial ischemia in diabetes: a quantitative analysis of angina perceptual threshold and the influence of autonomic function.J Am Coll Cardiol. 1990; 15:72–77.
34. Margolis JR, Kannel WS, Feinleib M, Dawber TR, McNamara PM.Clinical features of unrecognized myocardial infarction–silent and symptomatic. Eighteen year follow-up: the Framingham study. Am J Cardiol. 1973; 32:1–7.
35. Canto JG, Shlipak MG, Rogers WJ, Malmgren JA, Frederick PD, Lambrew CT, Ornato JP, Barron HV, Kiefe CI. Prevalence, clinical characteristics, and mortality among patients with myocardial infarction presenting without chest pain. JAMA. 2000; 283:3223–3229.
36. Shakespeare CF, Katritsis D, Crowther A, Cooper IC, Coltart JD, Webb-Peploe MW. Differences in autonomic nerve function in patients with silent and symptomatic myocardial ischaemia. Br Heart J. 1994; 71:22–29.
37. Rosen SD, Camici PG. The brain-heart axis in the perception of cardiac pain: the elusive link between ischaemia and pain. Ann Med. 2000; 32:350–364.

38. Wackers FJ, Young LH, Inzucchi SE, Chyun DA, Davey JA, Barrett EJ,Taillefer R, Wittlin SD, Heller GV, Filipchuk N, Engel S, Ratner RE, Iskandrian AE. Detection of silent myocardial ischemia in asymptomatic diabetic subjects: theDIAD study. Diabetes Care. 2004; 27:1954 –1961.
39. Stewart JM, Medow MS, Montgomery LD. Local vascular responses affecting blood flow in postural tachycardia syndrome. Am J Physiol Heart Circ Physiol. 2003; 285:H2749–H2756.
40. Stewart JM, Munoz J, Weldon A. Clinical and physiological effects of an acute alpha-1 adrenergic agonist and a beta-1 adrenergic antagonist in chronic orthostatic intolerance. Circulation. 2002; 106:2946 –2954.
41. Muenter Swift N, Charkoudian N, Dotson RM, Suarez GA, Low PA. Baroreflex control of muscle sympathetic nerve activity in the postural orthostatic tachycardia syndrome. Am J Physiol Heart Circ Physiol.2005; 289:H1226–1233.
42. Stewart JM, Glover JL, Medow MS. Increased plasma angiotensin II in Postural tachycardia syndrome (POTS) is related to reduced blood flow and blood volume. Clin Sci (Lond). 2006; 110:255–263.
43. Mustonen J, Uusitupa M, Lansimies E, et al. Autonomic nervous function and its relationship to cardiac performance in middle-aged diabetic patients without clinically evident cardiovascular disease. J Intern Med 1992; 232: 65–72.
44. Didangelos TP, Arsos GA, Karamitsos DT, et al. Left ventricular systolic and diastolic function in normotensive type 1 diabetic patients with or without autonomic neuropathy: a radionuclide ventriculography study. Diabetes Care 2003; 26: 1955–1960.

45. Lenzen MJ, Scholte op Reimer WJ, Boersma E, et al. Differences between patients with a preserved and a depressed left ventricular function: a report from the EuroHeart Failure Survey. Eur Heart J 2004; 25: 1214–1220.
46. Ansari M, Alexander M, Tutar A, et al. Incident cases of heart failure in a community cohort: importance and outcomes of patients with preserved systolic function. Am Heart J 2003; 146: 115–120.
47. Mandinov L, Eberli FR, Seiler C, et al. Diastolic heart failure. Cardiovasc Res 2000; 45: 813–825.
48. Perin PC, Maule S, Quadri R. Sympathetic nervous system, diabetes and hypertension. Clin Exp Hypertens 2001; 23: 45–55.
49. Valensi P, Sachs RN, Harfouche B, Lormeau B, Paries J, Cosson E.Predictive value of cardiac autonomic neuropathy in diabetic patients with or without silent mycardial ischemia. Diabetes Care. 2001; 24:339–343.
50. Veglio M, Borra M, Stevens LK, Fuller JH, Perin PC. The relation between QTc interval prolongation and diabetic complications: the EURODIAB IDDM Complications Study Group. Diabetologia. 1999; 42:68 –75.
51. Kahn JK, Sisson JC, Vinik AI. Prediction of sudden cardiac death in diabetic autonomic neuropathy. J Nucl Med. 1988; 29:1605–1606.
52. Stevens M, Dayanikli F, Raffel D, Allman K, Standford T, Feldman E,Wieland D, Corbett J, Schwaiger M. Scintigraphic assessment of regionalized defects in myocardial sympathetic intervation and blood flow regulation in diabetic patients with autonomic neuropathy. J Am Coll Cardiol. 1988; 31:1575–1584.
53. Toyry JP, Niskanen LK, Mantysaari MJ, Lansimies EA, Uusitupa MI.Occurrence, predictors, and clinical significance of autonomic neuropathy

in NIDDM: ten-year follow-up from the diagnosis. Diabetes. 1996; 45:308 – 315.

54. Miettinen H et al. Impact of diabetes on mortality after first myocardial infarction: the FINMONICA Myocardial Infarction Register Study Group. Diabetes Care.1998; 21:69 –75.
55. Katz A, Liberty IF, Porath A, Ovsyshcher I, Prystowsky EN. A simple bedside test of 1-minute heart rate variability during deep breathing as a prognostic index after myocardial infarction. Am Heart J. 1999; 138:32–38.
56. Wealtherall D.J., Ledingham J.G.G., Warrell D.A. Oxford textbook of Medicine, 3rd edn. Vol 3, sections 18-33 and index. Oxford university press inc., New York ; 1996. 3883 p.
57. Evaluation of autonomic functions in most centers pursuing autonomic investigations. Herkules. Oulu fi/isbn9514259637/html/i251817.html.21k.(accessed on 8/08/14).
58. Ziiegler Dan. Cardiovascular autonomic neuropathy: Clinical manifestations and measurements. Diabetic Review. 1999 ; 7 (4) : 342–357.
59. Malpas S. C and Maling T. J. D. Heart – Rate variability and cardiac autonomic function in diabetes. Diabetes. 1990 ; 39 : 1177 – 1181.
60. Ewing DJ, Martyn CN, Young RJ, Clarke BF. The value of cardiovascular function tests: 10 years experience in diabetes. Diabetes Care.1985; 8:491–498.
61. Ewing DJ. Autonomic function testing (clinical) – Michael Poon's Shrine of neurology. 1985, 1988 in www.Autonomic function testing.html. (accessed on 4/8/14).

62. LeBlance J, Dulac S, Cotej and Girard B. Autonomic Nervous System and adaptation to cold in man. Journal of Applied Physiology. 1975; 39 (2) : 181 – 186.
63. Singh K and Sood S. Autonomic Functions in Buerger's disease. Indian J Physiol Pharmacol. 2001; 45 (4) : 470 – 474.
64. Adams R. D. Victor M. Principle of Neurology. 4th edn. McGraw – Hill Inc; 1997, 431p.
65. Lee M.S, Lee J.S, Lee J.Y, Cornelissen G, Otsuka K, Halberg F. About 7-day (circaseptan) and circadian changes in cold pressor test (CPT). Biomed Pharmacother. 2003; 57 (1) : 39-44.
66. Autonomics, Heat rate variability, Blood pressure variability, and the baroreflex in www.nync.edu/…/ 20k (accessed on 4/8/14).
67. Ekta Khandelwal, Ashok Kumar Jaryal, Kishore Kumar Deepak. Pattern and Prevalence of Cardiovascular Autonomic Neuropathy in Diabetics visiting a Tertiary Care Referral Centre in India. Indian J Physiol Pharmacol 2011;55: 119-127.
68. Boulton AJ, Vinik AI, Arezzo JC, Bril V, Feldman EL, Freeman R, Malik RA, Maser RE, Sosenko JM, Ziegler D, American Diabetes Association. Diabetic neuropathies: a statement by the American Diabetes Association. Diabetes Care 2005; 28:956–962.
69. Assessment: clinical autonomic testing report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. Neurology 1996; 46:873–880.
70. Raffel DM, Wieland DM. Assessment of cardiac sympathetic nerve integrity with Positron emission tomography. Nucl MedBiol 2001; 28:541–559.

71. Stevens MJ, Raffle DM, Allman KC, Dayanikli F, Ficaro E, Sandford T, Wieland DM, Pfeifer MA, Schwaiger M. Cardiac sympathetic dysinnervation in diabetes:implications for enhanced cardiovascular risk. Circulation 1998; 98:961–968.
72. La Rovere MT, Bigger JT, Jr, Marcus FI, Mortara A, Schwartz PJ. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. Lancet 1998; 351:478–484.
73. Hamner JW, Taylor JA. Automated quantification of sympathetic beat-by-beat activity, independent of signal quality. J Appl Physiol 2001; 91:1199–206.
74. Vinik AI, Erbas T. Cardiovascular autonomic neuropathy: diagnosis and management. Curr Diab Rep 2006; 6:424–430.
75. Gaede P, Vedel P, Parving HH, et al. Intensified multifactorial intervention in patients with type 2 diabetes mellitus and microalbuminuria: the Steno type 2 randomized study. Lancet 1999; 353: 617–622.
76. Gaede P, Lund-Andersen H, Parving HH, et al. Effect of a multifactorial intervention on mortality in type 2 diabetes. N Engl J Med 2008; 358: 580–591.
77. Ziegler D, Schatz H, Conrad F, et al. Effects of treatment with the antioxidant alpha-lipoic acid on cardiac autonomic neuropathy in NIDDM patients. A 4-month randomized controlled multicenter trial (DEKAN Study). Deutsche Kardiale Autonome Neuropathie. Diabetes Care 1997; 20:369–373.
78. Tesfaye S, Boulton AJ, Dyck PJ, et al. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. Diabetes Care 2010; 33: 2285–2293.

79. Pop-Busui R, Evans G, Gerstein H, et al. , the ACCORD Study Group. Effects of cardiac autonomic dysfunction on mortality risk in the action to control cardiovascular risk in diabetes (ACCORD) trial. Diabetes Care 2010; 33: 1578–1584.
80. Bhatia S. G. Sainani S.G. Nayak N. J and Diwate P.G. Valsalva manoeuvre as a test of autonomic neuropathy in diabetes mellitus. Jr.Asso.Phys.Ind.1976 Feb; 24 : 89-93.
81. Ewings D.J, Campbell I.W, Clarke B.F. Heart rate changes in diabetes mellitus. The Lancet.1981 Jan ; 24: 183 – 185.
82. Maser RE, Pfeifer MA, Dorman JS, Kuller LH, Becker DJ, Orchard TJ. Diabetic autonomic neuropathy and cardiovascular risk. Pittsburgh Epidemiology of Diabetes complications study III. Arch Intern Med. 1990 June; 150(6) : 1218-22.
83. Selvin E, Marinopoulos S, Berkenblit G, Rami T, Brancati FL, Powel NR, et al. Meta- analysis: glycosylated hemoglobin and cardiovascular disease in diabetes mellitus. Ann Intern Med. 2004; 141:421-31.
84. Khaw KT, Wareham N, Bingham S, Luben R, Welch A, Day N. Association of hemoglobin A1c with cardiovascular disease and mortality in adults: the European Prospective Investigation into Cancer in Norfolk. Ann Intern Med. 2004; 141:413-20.
85. Unnikrishnan AG. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes. Lancet 1998; 352: 837-53.

86. Ghosh Asuthosh and Mukherjee Chandra Subhas. Evaluation of Autonomic Function in Diabetes mellitus. Ind. J. Physiol and Allied Sci. 1998; 52 (1) : 28 – 34.

87. Philips JC et al. Haemodynamic changes during a squat test, pulsatile stress and indices of cardiovascular autonomic neuropathy in patients with long-duration type 2 diabetes. Diabetes Metab. 2012 Feb;38(1):54-62. doi: 10.1016/j.diabet.2011.07.007.Epub 2011 Oct 24.

88. Ninković V, Ninković S, Zivojinović D. Cardiovascular autonomous dysfunction in diabetics: the influence of disease duration, glycoregulation degree and diabetes type. Srp Arh Celok Lek. 2008 Sep-Oct;136(9-10):488-93.

89. Valensi et al. Influence of blood glucose on heart rate and cardiac autonomic function. The DESIR study. Diabet Med. 2011 Apr;28(4):440-9. doi: 10.1111/j.1464-5491.2010.03222.

90. Ling DY, Tang ZY, Zhang W, Wu JC, Hou RF, Li XY, Wang WQ, Ning G.. Risk factors for cardiovascular autonomic neuropathy and their prognostic implications in type 2 diabetes mellitus. Zhonghua Nei Ke Za Zhi 2006 Oct; 45(10):815-9.

91. Ko SH et al. Progression of cardiovascular autonomic dysfunction in patients with type 2 diabetes: a 7-year follow-up study. Diabetes Care. 2008;31(9):1832.

92. Rodica Pop-Busui et al. Association Between Cardiovascular Autonomic Neuropathy and Left Ventricular Dysfunction DCCT/EDIC Study. J Am Coll Cardiol. 2013;61(4):447-454. doi:10.1016/j.jacc.2012.10.028.

93. Jae-Seung Yun, Ji-Hyun Kim, Ki-Ho Song. Cardiovascular Autonomic Dysfunction Predicts Severe Hypoglycemia in Patients With Type 2 Diabetes: A 10-Year Follow-up Study. Diabetes India 2014; 37 : 235-237.
94. M Charles, J Fleischer, D R White. Impact of early detection and treatment of diabetes on the 6 year prevalence of Cardiac Autonomic Neuropathy in people with screen – detected Diabetes. Diabetologia 2013; 56 : 101-108.
95. Didangelos T. Diabetic Cardiovascular Autonomic Neuropathy and Left Ventricular Dysfunction. General Med 2013; 34:12 - 14.
96. Tarvainen MP, Laitinen TP, Lipponen JA, Cornforth DJ, Jelinek HF. Cardiac autonomic dysfunction in type 2 diabetes - effect of hyperglycemia and disease duration. Front Endocrinol (Lausanne). 2014 ;5:130-132
97. Cardoso CR, Moraes RA, Leite NC, Salles GF. Relationships between reduced heart rate variability and pre-clinical cardiovascular disease in patients with type 2 diabetes. Diabetes Res Clin Pract. 2014; 106(1):110-7.
98. Tang ZH, Zeng F, Li Z, Zhou L. Association and predictive value analysis for resting heart rate and diabetes mellitus on cardiovascular autonomic neuropathy in general population. J Diabetes Res. 2014;2014:215473.
99. Tahrani AA, Dubb K, Raymond NT, Begum S, Altaf QA, Sadiqi H, Piya MK, Stevens MJ. Cardiac autonomic neuropathy predicts renal function decline in patients with type 2 diabetes: a cohort study. Diabetologia. 2014;57(6):1249-1256.
100. Fleischer J et al. Cardiovascular Autonomic Neuropathy Is Associated With Macrovascular Risk Factors in Type 2 Diabetes: New Technology Used for Routine Large-Scale Screening Adds New Insight. J Diabetes Sci Technol. 2014 Mar 31.

101. Aaron I. Vinik, Raelene E. Maser, Braxton D. Mitchell, Roy Freeman, “Diabetic Autonomic Neuropathy”. Diabetes Care May 2003; 26 :1553-1579.

102. J.M.Pappachan, J.Sebastian, B.C. Bino, et al, Cardiac Autonomic Neuropathy in Diabetes Mellitus: prevalence, risk factors and utility of Corrected QT interval in the ECG for its Diagnosis, Kottayam Medical College, South India. Melaka Manipal Medical College, Malaysia. 88. Rundle RW. "Diabetic neuropathy". Medicine (Baltimore) 1945; 24: 111-160.

103. A.Goel. Ruchika Agarwal, S.Singla, K.K. Lakhani, D.T. Sonigra, S.B. Agarwal, A clinical study on Autonomic nervous system manifestations in Diabetes mellitus. The Journal of the Association of Physicians of India 2005; 53: 997- 999.

104. Oluranti B.familoni, Olatunde odusan, Taiwo.H.Raimi, “The relationship between QT intervals and Cardiac Autonomic Neuropathy In Nigerian Patients with Type 2 Diabetes Mellitus”, Nigerian Medical Practioneer 2008; 53 : 48-51.

105. M. Lakhotia, P.K.D. Shah, R.Vyas, S.S. Jain, A. Yadav, M.K.Parihar, Clinical Dysautonomia in Diabetes Mellitus- A Study with Seven Autonomic Reflex Function Tests, JAPI 1997;45: 8-14.

106. Noronha J.L., Bhandarkar S.D., Shenoy P.N., Retnam V.J. Autonomic Neuropathy in Diabetes Mellitus, J Postgrad Med 1981; 27: 1-6.

107. Masaoka S, et al., "Heart rate variability in diabetes, relationship to age and duration of disease" Diabetologia 1980;18, 279-283.

108. Mohan V et al. Autonomic Neuropathy in NIDDM and fibrocalculus pancreatic diabetes in south India, Diabet Med 1996; 13: 1038-1043.

109. Aaron I. Vinik and Dan Ziegler: Diabetic Cardiovascular Autonomic Neuropathy. Circulation 2007; 115: 387-397.

110. Goldstein DE, Little RR, Lorenz RA, Malone JI, Nathan D, Peterson CM, Sacks DB. Diabetes care 2003; 27:1761-1773.

111. Peters AL. Peters MB. Davidson DL, Schriger V, Hasselblad. A clinical approach for the diagnosis of diabetes mellitus: an analysis using glycosylated hemoglobin levels. Meta-analysis Research Group on the Diagnosis of Diabetes Using Glycated Hemoglobin Levels J. Am. Med. Assoc., 1996; 276 :1246–1252.

112. Vinik A I, Maser E R, Mitchell B D, Freeman R:Diabetic autonomic neuropathy. Diabetes Care 2003; 26:1553-1579.

113. Ziegler D, Reljanovic M, Mehnert H, Gries FA: Alphalipoic acid in the treatment of diabetic polyneuropathy in Germany: current evidence from clinical trials.Exp Clin Endocrinol Diabetes1999; 107:421–430.

114. Kempler P, Tesfaye S, Chaturvedi N, Stevens L.K, Webb D.J, Eaton S, et al. Blood pressure response to standing in the diagnosis of autonomic neuropathy: The EURODIAB IDDM complications study. Archives of Physiology and Biochemistry. 2001 ; 109 (3) : 215 – 222.

115. Ewings D.J. cardiovascular reflexex and autonomic neuropathy. Clinical Science and Molecular Medicine. 1978; 55 : 321 – 327.

116. Popovic S, Todorovic Dilas L, Pantelinac P. The role of autonomic cardiovascular neuropathy in pathogenesis of ischemic heart disease in patients with diabetes mellitus. Med Pregl. 2006 Mar-Apr; 59(3-4):118-23.

117. Hines Edgar A and Brown George E. The cold pressor test for measuring the reactibility of the blood pressure: data concerning 571 normal and hypertensive subjects. The American Heart Journal. 1936 Jan; 11 (1): 1 – 9.

118. Sayinal P.S, Sozen T, Ozdogan M. cold pressor test in diabetic autonomic neuropathy. Diabetes. Res. Clin. Pract. 1994; 26 (1) : 21- 28.

119. Oikawa N, Umetsu M, Toyota T, Goto Y. Quantitative evaluation of diabetic autonomic neuropathy by using heart rate variations - determination of the normal range for the diagnosis of autonomic neuropathy. Tohoku J Exp Med. 1985; 145(3):233-41.

120. Sundvist G, Almer L- O, Lilja B. Respiratory influence on heart rate in diabetes mellitus. British Medical Journal. 1979; 1: 924 – 925.

121. Bhatia S. G. Sainani S.G. Nayak N. J and Diwate P.G. Valsalva manoeuvre as a test of autonomic neuropathy in diabetes mellitus. Jr.Asso.Phys.Ind.1976; 24 : 89-93.

122. Hague R, Scarpello J, Sladen G, Cullen D. Autonomic function tests in diabetes mellitus. Diabete Metab. 1978; 4(4):227-231.

123. Smith SA. Diagnostic value of the Valsalva ratio reduction in diabetic autonomic neuropathy: use of an age-related normal range. Diabet Med. 1984 Nov; 1(4):295-297.

ANNEXURES

1) Annexure I - PROFORMA FOR EVALUATION OF CARDIAC AUTONOMIC DYSFUNCTION IN TYPE 2 DIABETES (CASES)

A) DEMOGRAPHICS

1. Name
2. Age
3. Sex
4. Village/Town
5. Education
6. Smoker/Alcoholic

B) HISTORY OF TYPE 2 DIABETES

1. Age Of Onset Of Diabetes
2. Duration Of Diabetes
3. Family History Of Diabetes
4. Treatment for Diabetes

C) CLINICAL MANIFESTATIONS OF CARDIAC AUTONOMIC DYSFUNCTION

1. Exercise Intolerance
2. Orthostatic Hypotension

   (Dizziness/Presyncope)
3. Resting Tachycardia
4. Silent MI

D) Resting Heart Rate

SBP

DBP

E) INVESTIGATIONS – (CASES)

1)	FBS	
2)	PPBS	
3)	HbA1c	

F) CARDIAC AUTONOMIC FUNCTION TESTS

TESTS FOR SYMPATHETIC COMPONENT				
		MEASURED	CRITERIA	RESULT
1	Lying to Standing Test (LST) - (Change in SBP)		<10mm – N 11-20mm – Bo. >20 mm – Ab.	
2	Hand Grip Test (HGT) - (Change in DBP)		> 16mm – N 11 – 15mm – Bo. <10mm – Ab.	
3	Cold Pressor Test (CPT) - (Change in DBP)		> 16mm – N 11 – 15mm – Bo. <10mm – Ab.	
TESTS FOR PARASYMPATHETIC COMPONENT				
		MEASURED	CRITERIA	RESULT
1	Deep Breathing Test **(DBT)** - (Delta Heart Rate)		>15bpm – N. 11-14bpm – Bo. <10bpm – Ab.	
2	Valsalva Maneuver **(Valsalva ratio) – (VR)**		>1.21 – N. 1.11 – 1.20 – Bo. <1.10 – Ab.	
3	Lying to Standing **(30: 15 ratio)**		>1.04 – N. 1.01 – 1.03 – Bo. <1.01 – Ab.	

Annexure II

PROFORMA FOR EVALUATION OF CARDIAC AUTONOMIC DYSFUNCTION IN NON DIABETIC INDIVIDUALS (CONTROLS)

A) DEMOGRAPHICS

1. Name
2. Age
3. Sex
4. Village/Town
5. Education
6. Smoker/Alcoholic

B) CLINICAL MANIFESTATIONS OF CARDIAC AUTONOMIC DYSFUNCTION

1. Exercise Intolerance
2. Orthostatic Hypotension (Dizziness/Presyncope)
3. Resting Tachycardia
4. Silent MI

C) Resting Heart Rate

SBP

DBP

F) INVESTIGATIONS – (CONTROLS)

4) FBS	
5) PPBS	
6) HbA1c	

G) CARDIAC AUTONOMIC FUNCTION TESTS

TESTS FOR SYMPATHETIC COMPONENT				
		MEASURED	CRITERIA	RESULT
1	Lying to Standing Test (LST) - (Change in SBP)		<10mm – N 11-20mm – Bo. >20 mm – Ab.	
2	Hand Grip Test (HGT) - (Change in DBP)		> 16mm – N 11 – 15mm – Bo. <10mm – Ab.	
3	Cold Pressor Test (CPT) - (Change in DBP)		> 16mm – N 11 – 15mm – Bo. <10mm – Ab.	
TESTS FOR PARASYMPATHETIC COMPONENT				
		MEASURED	CRITERIA	RESULT
1	Deep Breathing Test **(DBT)** - (Delta Heart Rate)		>15bpm – N. 11-14bpm – Bo. <10bpm – Ab.	
2	Valsalva Maneuver **(Valsalva ratio) – (VR)**		>1.21 – N. 1.11 – 1.20 – Bo. <1.10 – Ab.	
3	Lying to Standing **(30: 15 ratio)**		>1.04 – N. 1.01 – 1.03 – Bo. <1.01 – Ab.	

ANNEXURE III

Patient details:

Name: **Age:** **Gender:** **Hospital No.**

Title of the Study: STUDY OF CARDIAC AUTONOMIC NEUROPATHY IN TYPE 2 DIABETES MELLITUS

INFORMED CONSENT

I, _____, exercising my free power of choice, hereby give my consent to be included as a subject in the **"STUDY OF CARDIAC AUTONOMIC NEUROPATHY IN TYPE 2 DIABETES MELLITUS "** under the principal investigatorship of **Dr. A.N.L.N Murthy.** I understand that I remain free to withdraw from this study at any time. I have read or had read to me and understand the purpose of this study and the confidential nature of the information that will be collected and disclosed during the study. I have had the opportunity to ask my questions regarding the various aspects of this study and my questions have been answered to my satisfaction. I, the undersigned agree to participate in this study and authorize the collection and disclosure of my personal information as outlined in this consent form.

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Participant's Name & signature Date

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Signature of the witness Date

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Signature of the principal investigator Date

ANNEXURE IV

1) Recording of Delta Heart Rate in Deep Breathing Test

2) Recording rise in DBP in Cold Pressor Test.

3) Recording of Lying to Standing Test (30:15 Ratio)

4) Recording of Valsalva ratio

MASTER CHART

S.No	Groups	Groupscoded	Sex	Age	Duration of T2DM	FBS	PPBS	HbA1c	RHR	SBP	DBP	LST(ΔSBP in mm)	LST -Result	HGT(ΔDBP in mm)	HGT-Result	CPT(ΔDBP in mm)	CPT-Res	DBT (δHR)	DBT-Result	VR	VR-Result	30:15R	30:15R-Result	CAN
1	Cases	1	M	47	4	124	146	6.92	73	130	74	5	N	17	N	16	N	17	N	1.22	N	1.07	N	Absent
2	Cases	1	M	52	8	142	167	7.42	86	140	70	12	BO	18	N	17	N	8	BO	1.23	N	1.04	N	Early CAN
3	Cases	1	F	42	2	96	122	6.35	74	132	60	4	N	18	N	16	N	21	N	1.22	N	1.08	N	Absent
4	Cases	1	M	59	6	132	176	8.24	64	140	74	14	BO	16	N	13	BO	8	AB	1.06	AB	1	AB	Definite CAN
5	Cases	1	F	55	6	86	107	6.04	68	140	84	6	N	16	N	14	BO	16	N	1.25	N	1.02	BO	Early CAN
6	Cases	1	M	41	3	132	182	7.94	76	124	80	22	AB	13	BO	16	N	17	N	0.98	AB	1.02	BO	Definite CAN
7	Cases	1	F	54	7	140	176	8.34	58	140	80	6	N	8	AB	17	N	13	BO	1.03	AB	1	N	Definite CAN
8	Cases	1	M	41	1	76	106	6.04	80	128	76	3	N	16	N	20	N	18	N	1.26	N	1	N	Absent
9	Cases	1	M	61	12	176	194	9.26	58	150	88	24	AB	16	N	21	N	18	N	1.33	N	0.92	AB	Definite CAN
10	Cases	1	M	45	4	83	122	6.56	74	130	82	7	N	18	N	21	N	19	N	1.62	N	1.03	BO	Absent
11	Cases	1	M	47	6	98	144	6.34	74	130	80	12	BO	16	N	20	N	23	N	1.43	N	1.06	N	Absent
12	Cases	1	F	69	12	102	107	7.12	62	144	4	8	N	6	AB	16	N	12	AB	1.14	BO	1.07	N	Definite CAN
13	Cases	1	M	43	6	78	96	5.96	78	120	80	6	N	16	N	16	N	17	N	1.67	N	1.04	N	Absent
14	Cases	1	M	53	3	201	254	10.34	69	134	76	24	AB	18	N	19	N	9	AB	2.33	N	1.02	BO	Definite CAN
15	Cases	1	F	64	10	156	203	7.65	64	146	78	12	BO	13	BO	21	N	21	N	1.14	BO	1.04	N	Early CAN
16	Cases	1	M	49	4	112	134	6.56	68	130	68	14	BO	12	BO	21	N	22	N	2.12	N	1.06	N	Absent
17	Cases	1	M	55	9	145	176	8.12	70	144	70	8	N	17	N	20	N	12	BO	1.18	BO	1.07	N	Early CAN
18	Cases	1	F	42	3	98	123	6.79	62	110	60	6	N	18	N	19	N	16	N	1.56	N	1.06	N	Absent
19	Cases	1	M	58	7	188	223	8.22	64	134	68	14	BO	8	AB	6	AB	19	N	1.24	N	1.05	N	Definite CAN
20	Cases	1	F	41	8	102	156	6.23	68	130	74	6	N	16	N	16	N	17	N	1.37	N	1.05	N	Absent
21	Cases	1	M	63	14	134	176	7.56	56	144	64	14	BO	12	BO	17	N	7	AB	1.32	N	0.96	AB	Definite CAN
22	Cases	1	M	47	6	122	156	6.35	70	130	80	4	N	16	N	17	N	22	N	2.33	N	1.04	N	Absent
23	Cases	1	M	56	12	232	282	9.4	64	140	84	8	N	18	N	18	N	5	AB	1.16	AB	1.03	BO	Definite CAN
24	Cases	1	M	44	2	110	154	6.72	76	126	76	8	N	19	N	16	N	19	N	1.67	N	1.04	N	Absent
25	Cases	1	M	45	7	176	202	7.56	72	130	80	4	N	20	N	16	N	24	N	1.92	N	1.02	BO	Early CAN
26	Cases	1	M	52	4	98	114	6.26	64	140	76	4	N	22	N	19	N	22	N	1.34	N	1.06	N	Absent

MASTER CHART

S.No	Groups	Groupscoded	Sex	Age	Duration of T2DM	FBS	PPBS	HbA1c	RHR	SBP	DBP	LST(ΔSBP in mm)	LST -Result	HGT(ΔDBP in mm)	HGT-Result	CPT(ΔDBP in mm)	CPT-Res	DBT (δHR)	DBT-Result	VR	VR-Result	30:15R	30:15R-Result	CAN
27	Cases	1	F	48	7	134	176	7.24	68	140	88	12	BO	16	N	8	AB	8	AB	1.24	N	1.03	BO	Definite CAN
28	Cases	1	M	45	1	177	255	9.45	66	134	76	6	N	16	N	17	N	12	BO	1.14	BO	1.04	N	Early CAN
29	Cases	1	M	50	6	134	176	6.94	70	140	80	6	N	18	N	16	N	23	N	1.54	N	1.04	N	Absent
30	Cases	1	F	45	2	112	156	7.56	62	130	70	14	BO	13	BO	16	N	28	N	1.67	N	1.12	N	Early CAN
31	Cases	1	M	52	6	109	199	7.33	56	140	90	22	AB	22	N	18	N	19	N	1.12	AB	1.07	N	Definite CAN
32	Cases	1	M	42	6	123	156	6.27	64	114	60	4	N	21	N	16	N	17	N	1.94	N	1.06	N	Absent
33	Cases	1	F	57	13	143	185	7.23	66	134	68	13	BO	21	N	17	N	16	N	1.33	N	1.04	N	Absent
34	Cases	1	M	54	3	155	223	8.24	69	140	78	8	N	6	AB	20	N	16	N	1.67	N	1.05	N	Early CAN
35	Cases	1	F	67	16	165	244	7.76	72	144	80	12	BO	20	N	21	N	16	N	1.12	AB	0.94	AB	Definite CAN
36	Cases	1	M	58	12	198	301	8.43	76	138	84	12	BO	18	N	22	N	9	AB	1.14	AB	1.06	N	Definite CAN
37	Cases	1	F	42	3	111	212	7.64	80	120	70	6	N	14	AB	9	AB	19	N	1.67	N	1.06	N	Definite CAN
38	Cases	1	F	62	12	154	180	8.34	66	150	84	22	AB	16	N	12	BO	22	N	1.16	AB	0.98	AB	Definite CAN
39	Cases	1	M	54	4	167	266	7.77	62	140	76	8	N	18	N	22	N	24	N	1.09	AB	1.04	N	Early CAN
40	Cases	1	M	46	6	76	98	6.5	64	136	80	6	N	16	N	24	N	22	N	2.52	N	1.07	N	Absent
41	Cases	1	F	62	11	276	334	10.94	58	140	78	12	BO	16	N	16	N	22	N	1.17	AB	0.96	AB	Definite CAN
42	Cases	1	F	54	8	123	143	6.34	62	124	80	7	N	18	N	18	N	19	N	1.33	N	1.06	N	Absent
43	Cases	1	M	45	6	156	176	7.42	60	120	76	6	N	19	N	16	N	17	N	1.67	N	1.05	N	Absent
44	Cases	1	F	56	13	179	196	7.96	63	140	78	6	N	16	N	14	BO	8	AB	1.17	AB	1.05	N	Definite CAN
45	Cases	1	M	56	10	205	376	9.22	62	134	88	14	BO	7	AB	16	N	11	BO	1.18	AB	1.06	N	Definite CAN
46	Cases	1	F	51	9	85	114	6.45	64	132	76	4	N	16	N	17	N	19	N	2.52	N	1.04	N	Absent
47	Cases	1	F	42	3	134	176	7.92	72	120	70	12	BO	16	N	18	N	8	AB	2.67	N	1.07	N	Early CAN
48	Cases	1	M	62	6	82	122	5.76	64	156	84	8	N	19	N	16	N	21	N	2.45	N	1.07	N	Absent
49	Cases	1	F	53	9	112	159	7.34	70	146	76	8	N	20	N	16	N	21	N	2.22	N	1.08	N	Absent
50	Cases	1	F	64	12	134	176	8.32	62	140	70	8	N	9	AB	20	N	6	AB	1.23	N	0.93	AB	Definite CAN
51	Cases	1	M	57	9	88	96	6.09	64	132	70	13	BO	18	N	22	N	21	N	1.54	N	1.02	BO	Early CAN
52	Cases	1	F	62	12	124	161	7.54	64	154	88	12	BO	13	BO	21	N	18	N	1.16	AB	0.93	AB	Definite CAN

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S.No	Groups	Groupscoded	Sex	Age	Duration of T2DM	FBS	PPBS	HbA1c	RHR	SBP	DBP	LST(ΔSBP in mm)	LST -Result	HGT(ΔDBP in mm)	HGT-Result	CPT(ΔDBP in mm)	CPT-Res	DBT (δHR)	DBT-Result	VR	VR-Result	30:15R	30:15R-Result	CAN
53	Cases	1	M	59	9	76	109	5.34	60	140	78	4	N	18	N	20	N	18	N	1.67	N	1.04	N	Absent
54	Cases	1	F	52	8	170	227	7.66	58	140	78	8	N	16	N	20	N	8	AB	1.98	N	1.08	N	Early CAN
55	Cases	1	M	49	9	68	98	5.78	62	134	76	4	N	16	N	16	N	16	N	1.34	N	1.07	N	Absent
56	Cases	1	M	53	7	110	167	7.23	54	122	68	6	N	16	N	18	N	23	N	1.67	N	1.06	N	Absent
57	Cases	1	F	47	5	112	136	6.34	72	128	78	6	N	16	N	18	N	13	BO	2.67	N	1.04	N	Absent
58	Cases	1	M	56	10	198	264	7.43	64	140	80	22	AB	19	N	17	N	21	N	1.34	N	1.05	N	Early CAN
59	Cases	1	F	62	12	134	201	8.32	66	146	90	8	N	5	AB	8	AB	22	N	1.45	N	1.06	N	Definite CAN
60	Cases	1	M	54	6	103	145	6.76	56	124	80	4	N	17	N	16	N	17	N	1.92	N	1.05	N	Absent
61	Controls	2	F	48		72	110	5.74	74	124	82	6	N	18	N	20	N	20	N	1.32	N	1.08	N	Absent
62	Controls	2	M	43		91	124	5.8	68	134	80	13	BO	20	N	16	N	18	N	1.4	N	1.06	N	Absent
63	Controls	2	F	54		104	136	4.92	66	124	76	6	N	17	N	18	N	16	N	1.26	N	1.12	N	Absent
64	Controls	2	F	62		102	124	5.92	64	110	70	8	N	18	N	18	N	24	N	1.22	N	1.08	N	Absent
65	Controls	2	M	57		65	84	5.6	76	120	76	4	N	20	N	16	N	6	AB	1.23	N	1.14	N	Early CAN
66	Controls	2	F	54		88	98	5.34	69	132	80	4	N	22	N	22	N	16	N	1.42	N	1.06	N	Absent
67	Controls	2	M	61		124	148	5.76	60	112	88	2	N	16	N	18	N	28	N	1.45	N	1.07	N	Absent
68	Controls	2	F	47		92	108	5.4	79	130	80	7	N	17	N	22	N	24	N	1.24	N	1.21	N	Absent
69	Controls	2	M	43		73	96	4.8	84	114	74	6	N	23	N	24	N	16	N	1.27	N	1.15	N	Absent
70	Controls	2	F	51		93	108	5.62	76	150	76	5	N	18	N	18	N	19	N	1.35	N	1.16	N	Absent
71	Controls	2	M	68		64	93	5.79	68	160	90	6	N	18	N	20	N	16	N	1.23	N	1.23	N	Absent
72	Controls	2	F	44		106	116	6	76	140	70	6	N	19	N	16	N	20	N	1.26	N	1.07	N	Absent
73	Controls	2	M	59		112	142	5.21	60	94	60	8	N	22	N	16	N	22	N	1.34	N	1.11	N	Absent
74	Controls	2	F	64		93	106	5.43	78	130	70	4	N	20	N	13	BO	28	N	1.14	BO	1.07	N	Early CAN
75	Controls	2	F	43		72	94	5.6	82	124	74	4	N	18	N	20	N	16	N	1.34	N	1.06	N	Absent
76	Controls	2	F	50		85	96	5.12	66	140	86	5	N	16	N	20	N	18	N	1.24	N	1.05	N	Absent
77	Controls	2	M	70		89	94	5.34	74	130	90	6	N	16	N	6	AB	18	BO	1.06	AB	1.13	N	Definite CAN
78	Controls	2	F	54		90	112	5.67	72	140	84	4	N	18	N	17	N	16	N	1.32	N	1.04	N	Absent

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S.No	Groups	Groupscoded	Sex	Age	Duration of T2DM	FBS	PPBS	HbA1c	RHR	SBP	DBP	LST(ΔSBP in mm)	LST -Result	HGT(ΔDBP in mm)	HGT-Result	CPT(ΔDBP in mm)	CPT-Res	DBT (δHR)	DBT-Result	VR	VR-Result	30:15R	30:15R-Result	CAN
79	Controls	2	M	67		98	132	4.96	66	136	76	4	N	14	BO	16	N	24	N	1.33	N	1.12	N	Absent
80	Controls	2	F	56		106	128	5.6	68	120	78	6	N	18	N	16	N	18	N	1.26	N	1.06	N	Absent
81	Controls	2	M	62		102	144	5.6	64	114	78	17	BO	16	N	18	N	18	N	1.43	N	1.08	N	Absent
82	Controls	2	F	52		112	132	5.8	72	146	90	8	N	19	N	19	N	24	N	1.53	N	1.05	N	Absent
83	Controls	2	M	41		74	104	5.34	88	132	80	4	N	17	N	21	N	18	N	1.67	N	1.04	N	Absent
84	Controls	2	F	61		99	117	5.96	60	134	84	8	N	19	N	22	N	24	N	1.32	N	1.05	N	Absent
85	Controls	2	M	52		92	128	5.67	70	112	70	8	N	22	N	20	N	20	N	1.56	N	1.09	N	Absent
86	Controls	2	F	59		92	143	5.96	64	126	76	4	N	17	N	24	N	12	BO	1.76	N	1.02	BO	Early CAN
87	Controls	2	F	44		72	88	5.34	76	130	90	15	BO	19	N	18	N	22	N	1.34	N	1.2	N	Absent
88	Controls	2	M	55		109	132	5.9	64	100	70	4	N	21	N	16	N	24	N	1.4	N	1.12	N	Absent
89	Controls	2	F	51		79	95	5.54	70	90	60	6	N	20	N	16	N	20	N	1.7	N	1.09	N	Absent
90	Controls	2	F	49		82	101	5.43	74	122	80	6	N	12	BO	16	N	18	N	1.15	BO	1.12	N	Early CAN
91	Controls	2	M	45		116	134	5.78	78	140	84	3	N	17	N	16	N	16	N	1.34	N	1.09	N	Absent
92	Controls	2	M	62		93	116	5.34	66	122	78	6	N	18	N	18	N	18	N	1.23	N	1.08	N	Absent
93	Controls	2	F	63		105	137	4.46	68	126	76	8	N	19	N	19	N	8	AB	1.02	AB	1.01	BO	Definite CAN
94	Controls	2	M	46		76	93	5.79	76	130	84	8	N	18	N	17	N	16	N	1.34	N	1.24	N	Absent
95	Controls	2	M	42		111	122	5.34	78	124	76	5	N	18	N	17	N	24	N	1.56	N	1.07	N	Absent
96	Controls	2	M	53		69	87	5.04	78	110	70	4	N	16	N	16	N	22	N	1.67	N	1.09	N	Absent
97	Controls	2	F	41		102	124	5.34	84	110	90	3	N	16	N	14	BO	20	N	1.34	N	1.11	N	Absent
98	Controls	2	M	56		94	144	5.95	64	112	60	8	N	20	N	16	N	14	BO	1.18	BO	1.22	N	Early CAN
99	Controls	2	F	52		73	138	5.76	64	146	88	4	N	16	N	20	N	22	N	1.22	N	1.06	N	Absent
100	Controls	2	M	45		65	90	4.86	72	94	70	8	N	18	N	18	N	26	N	1.29	N	1.05	N	Absent
101	Controls	2	M	51		82	102	4.44	80	120	76	6	N	19	N	18	N	24	N	1.33	N	1.06	N	Absent
102	Controls	2	F	52		70	132	5.32	56	124	80	4	N	20	N	18	N	28	N	1.67	N	1.08	N	Absent
103	Controls	2	M	45		86	122	5.65	84	110	60	6	N	18	N	20	N	26	N	1.54	N	1.14	N	Absent
104	Controls	2	M	46		101	123	5.43	79	140	78	6	N	21	N	18	N	32	N	1.92	N	1.09	N	Absent

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S.No	Groups	Groupscoded	Sex	Age	Duration of T2DM	FBS	PPBS	HbA1c	RHR	SBP	DBP	LST(ΔSBP in mm)	LST -Result	HGT(ΔDBP in mm)	HGT-Result	CPT(ΔDBP in mm)	CPT-Res	DBT (δHR)	DBT-Result	VR	VR-Result	30:15R	30:15R-Result	CAN
105	Controls	2	F	42		76	101	5.78	75	120	80	4	N	17	N	17	N	20	N	1.34	N	1.17	N	Absent
106	Controls	2	M	59		79	122	5.44	64	110	76	8	N	18	N	22	N	24	N	1.45	N	1.23	N	Absent
107	Controls	2	M	43		101	146	5.9	74	104	68	4	N	16	N	20	N	18	N	1.93	N	1.24	N	Absent
108	Controls	2	M	56		84	98	5.34	64	100	70	7	N	16	N	14	BO	16	N	1.65	BO	1.05	N	Absent
109	Controls	2	F	52		76	85	4.85	69	144	90	5	N	18	N	16	N	16	N	1.3	N	1.04	N	Absent
110	Controls	2	M	44		67	83	5.67	64	146	86	3	N	19	N	16	N	18	N	1.45	N	1.22	N	Absent
111	Controls	2	M	48		101	134	5.39	76	110	72	7	N	20	N	16	N	18	N	1.4	N	1.06	N	Absent
112	Controls	2	M	51		95	112	5.93	72	132	74	8	N	18	N	18	N	16	N	1.27	N	1.04	N	Absent
113	Controls	2	M	57		67	86	5.43	76	144	76	5	N	17	N	24	N	24	N	1.45	N	1.06	N	Absent
114	Controls	2	M	43		92	109	5.67	88	126	90	5	N	19	N	20	N	22	N	1.67	N	1.07	N	Absent
115	Controls	2	M	57		69	111	5.32	64	134	80	7	N	16	N	24	N	20	N	1.04	AB	1.06	N	Early CAN
116	Controls	2	M	52		76	95	5.79	60	128	70	4	N	18	N	28	N	28	N	1.34	N	1.06	N	Absent
117	Controls	2	M	45		74	105	5.36	66	140	74	4	N	16	N	18	N	12	BO	1.6	N	1.04	N	Absent
118	Controls	2	M	48		106	121	5.04	70	144	84	7	N	20	N	16	N	18	N	1.34	N	1.12	N	Absent
119	Controls	2	M	54		115	144	5.32	74	132	88	6	N	18	N	18	N	16	N	1.5	N	1.26	N	Absent
120	Controls	2	M	47		93	107	5.64	72	126	70	6	N	16	N	16	N	12	BO	1.67	N	1.08	N	Absent