Detection of autonomic neuropathy in diabetic patients with peripheral neuropathy and its correlation with disease severity

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CERTIFICATE

I, Dr. Ajith Cherian hereby declare that I have actually carried out the project under report.

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CONTENTS

INTRODUCTION

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 (1). A subtype of the peripheral polyneuropathies that accompany diabetes, DAN can involve the entire autonomic nervous system (ANS). DAN may be either clinically evident or subclinical. It is manifested by dysfunction of one or more organ systems. Many organs are dually innervated, receiving fibers from the parasympathetic and sympathetic divisions of the ANS. DAN typically occurs as a system-wide disorder affecting all parts of the ANS because the vagus nerve (the longest of the ANS nerves) accounts for 75% of all parasympathetic activity (2), and DAN manifests first in longer nerves. Even early effects of DAN can be widespread.

Clinical symptoms of autonomic neuropathy generally do not occur until long after the onset of diabetes. Whereas symptoms suggestive of autonomic dysfunction may be common they may frequently be due to other causes rather than to true autonomic neuropathy. Subclinical autonomic dysfunction can, however, occur within a year of diagnosis in type 2 diabetes patients and within two years in type 1diabetes patients (3). Because of its association with a variety of adverse outcomes including cardiovascular deaths, cardiovascular autonomic neuropathy (CAN) is the most clinically important and well-studied form of DAN. The introduction over 20 years ago of simple, noninvasive tests of cardiovascular autonomic function has supported extensive clinical and epidemiologic investigation of CAN. These data form the strongest body of evidence for the importance of detecting and monitoring impaired autonomic function in patients with diabetes

The Chennai Urban Population Study (4) has shown that the incidence rate of diabetes was 20.2 per 1000 person years among subjects with normal glucose tolerance. The study showed that the incidence of diabetes is very high among urban south Indians. Autonomic neuropathy is frequent in

diabetics. Some evidence of it was found in 26 out of 33 (78.8%) patients in study done by Noronha et al (5). Bhatia et al (6) found evidence for autonomic neuropathy (either symptoms or an abnormal response to Valsalva manoeuvre or both) in 29 out of 100 patients they studied. The current study was carried out to evaluate patients with diabetic peripheral neuropathy and to assess evidence of autonomic dysfunction by means of simple, bedside tests and to stratify patients based on the classification proposed by Ewing et al. Also efforts were made to assess correlation between autonomic neuropathy with diabetes disease duration, glycemic control, stage of diabetic peripheral neuropathy and with other end organ damage due to diabetes mellitus especially with coronary artery disease.

REVIEW OF LITERATURE

Pathogenesis Of Diabetic Autonomic Neuropathy

Hypotheses concerning the multiple etiologies of diabetic neuropathy include a metabolic insult to nerve fibers, neurovascular insufficiency, autoimmune damage, and neurohormonal growth factor deficiency (7). Several different factors have been implicated in this pathogenic process. Hyperglycemic activation of the polyol pathway leading to accumulation of sorbitol and potential changes in the NAD: NADH ratio may cause direct neuronal damage and/or decreased nerve blood flow (8,9). Activation of protein kinase C induces vasoconstriction and reduces neuronal blood flow (10). Increased oxidative stress, with increased free radical production, causes vascular endothelium damage and reduces nitric oxide bioavailability (11,12). Alternately, excess nitric oxide production may result in formation of peroxynitrite and damage endothelium and neurons, a process referred to as nitrosative stress (13,14). In a subpopulation of individuals with neuropathy, immune mechanisms may also be involved (15). Reduction in neurotrophic growth factors (16), deficiency of essential fatty acids (17), and formation of advanced glycosylation end products (localized in endoneurial blood vessels) (18) also result in reduced endoneurial blood flow and nerve hypoxia with altered nerve function (7). The result of this multifactorial process may be activation of polyADP ribosylation depletion of ATP, resulting in cell necrosis and activation of genes involved in neuronal damage (19).

Epidemiology Of Diabetic Autonomic Neuropathy

The reported prevalence of diabetic autonomic neuropathy (DAN) varies, depending on whether studies have been carried out in the community, clinic, or tertiary referral center. The variance among prevalence studies also reflects the type and number of tests performed and the presence or absence of signs and symptoms of autonomic neuropathy. Other factors that account for the

marked variability in reported prevalence rates include the lack of a standard accepted definition of DAN, different diagnostic methods, variable study selection criteria, and referral bias (20). Additional complicating factors include the wide variety of clinical syndromes and confounding variables such as age, sex, duration of diabetes, glycemic control, diabetes type, height, and other factors. Table 1 reveals the prevalence rates of CAN for several different studies, again indicating the dramatic variability from a low of 7.7% for newly diagnosed patients with type 1 diabetes, when strict criteria to define CAN were used (20), to a high of 90% in potential recipients of a pancreas transplant (21). **Table 1**

Author	Date of publication	Diabetes type	Subjects (*n*)	Test(s) used	% Abnormal
Sharpey-Schafer and Taylor (22)	1960	Mixed	337	Valsalva maneuver	21
Ewing et al. (23)	1974	Mixed with autonomic symptoms	124	Handgrip test	18
Morley et al. (24)	1977	Adult diabetic patients	70	Valsalva maneuver	24
				Heart rate variation	11
Hilsted and Jensen (25)	1979	Insulin-treated	126	Heart rate variation	40
Mackay et al. (26)	1980	Mixed	287	Heart rate variation	30
Ewing et al. (27)	1980	Mixed with autonomic symptoms	73	Valsalva maneuver	47
				Handgrip	35
				Postural BP	45

A number of studies have been conducted to assess the prevalence of DAN in defined populations.

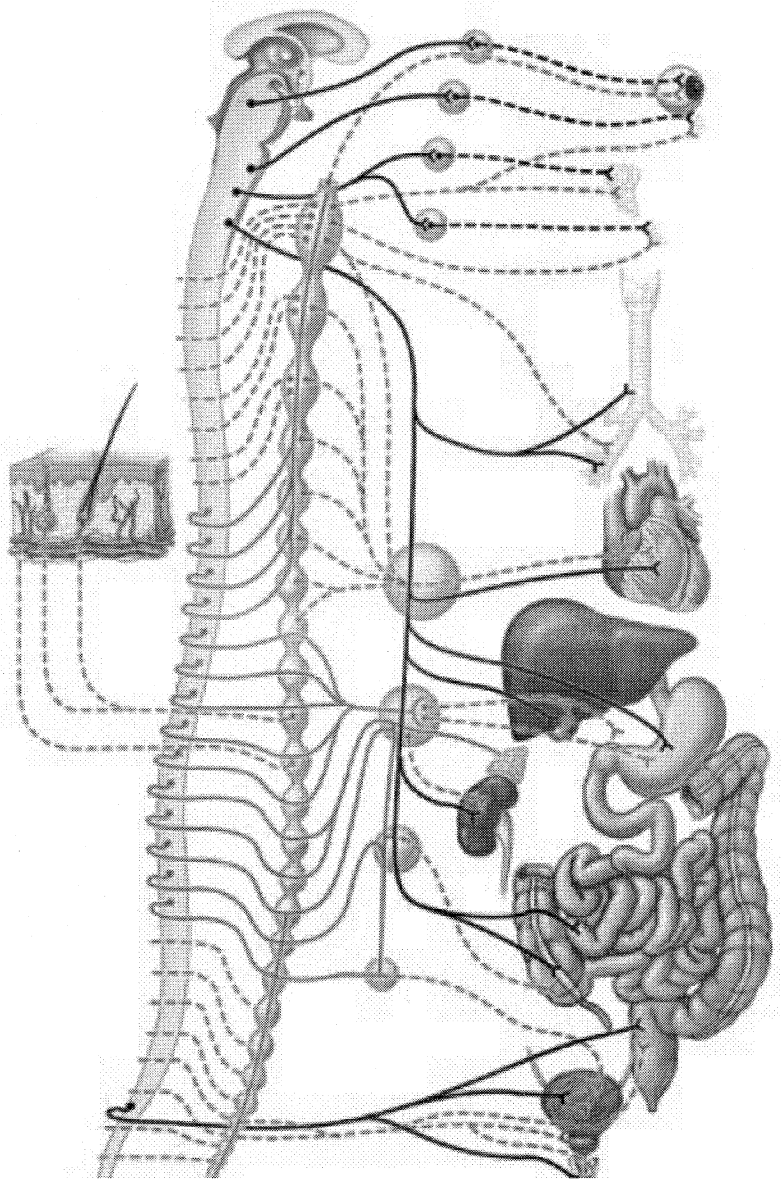
In a community-based population study of diabetic neuropathy in Oxford, England, the prevalence of autonomic neuropathy as defined by one or more abnormal heart rate variability (HRV) test results was 16.7% (28). In a further study, Ziegler et al. (20) evaluated the prevalence of CAN in 1,171 diabetic patients (647 type 1 diabetic patients, 524 type 2 diabetic patients) randomly recruited from 22 diabetes centers in Germany, Austria, and Switzerland. The study found that 25.3% of patients with type 1 diabetes and 34.3% of patients with type 2 diabetes had abnormal findings in more than two of six autonomic function tests. If more strict criteria were used (i.e., abnormalities present in least three of six autonomic function tests), the prevalence of CAN was 16.8% for individuals with type 1 diabetes and 22.1% for individuals with type 2 diabetes. Another study group observed nearly an identical prevalence rate (16.6%) for individuals with insulin-dependent diabetes (29).

Additional studies suggest that the prevalence of DAN may be even more common than these studies report. For example, using a variety of simple, validated, and noninvasive tests (e.g., fall in systolic blood pressure and heart rate response after standing), Verrotti et al. (30) found that 47 of 110 diabetic children and adolescents showed one or more abnormal tests for cardiovascular autonomic dysfunction. These results, however, recapitulate that prevalence rates will vary depending on *1*) different patient cohorts studied, *2*) varied testing modalities utilized, and *3*) different criteria used to define autonomic dysfunction.

CLINICAL MANIFESTATIONS OF DAN

The metabolic disorders of diabetes lead to diffuse and widespread damage of peripheral nerves and small vessels. Clinical manifestations of autonomic dysfunction and other microvascular complications frequently occur concurrently but in inconsistent patterns (31). The ubiquitous distribution of the

ANS renders virtually all organs susceptible to autonomic dysfunction. Therefore, a patient diagnosed with diabetes should be suspected of having at least subclinical disturbances of the ANS. Overt signs and symptoms of autonomic disease fall into one or more of the following categories.



Pupillary
Decreased diameter of dark-adapted pupil
Argyll-Robertson type pupil

Metabolic
Hypoglycemia unawareness
Hypoglycemia unresponsiveness

Cardiovascular
Tachycardia, exercise intolerance
Cardiac denervation
Orthostatic hypotension
Heat intolerance

Neurovascular
Areas of symmetrical anhydrosis
Gustatory sweating
Hyperhidrosis
Alterations in skin blood flow

Gastrointestinal
Constipation
Gastroparesis diabeticorum
Diarrhea and fecal incontinence
Esophageal dysfunction

Genitourinary
Erectile dysfunction
Retrograde ejaculation
Cystopathy
Neurogenic bladder
Defective vaginal lubrication

The differential diagnosis of DAN involves excluding the following conditions:

- Pure autonomic failure (formerly called idiopathic orthostatic hypotension)

- Multiple system atrophy with autonomic failure (formerly called Shy-Drager syndrome)
- Addison's disease and hypopituitarism
- Pheochromocytoma
- Hypovolemia
- Medications, with anticholinergic or sympatholytic effects (insulin, vasodilators, sympathetic blockers)
- Peripheral autonomic neuropathies (e.g., amyloid neuropathy, idiopathic autonomic neuropathy)

DAN is typically assessed by focusing on symptoms or dysfunction attributable to a specific organ system. CAN is the most prominent focus because of the life-threatening consequences of this complication and the availability of direct tests of cardiovascular autonomic function. However, neuropathies involving other organ systems should also be considered in the optimal care of patients with diabetes.

Cardiovascular Autonomic Neuropathy (CAN)

Perhaps one of the most overlooked of all serious complications of diabetes is CAN (32). 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 (33). Reduced heart rate variation is the earliest indicator of CAN (34). In a review of several epidemiological studies among individuals diagnosed with diabetes, it was shown that the 5-year mortality rate from this serious complication is five times higher for individuals with CAN than for individuals without cardiovascular autonomic involvement (3).

Clinical manifestations of CAN

Exercise intolerance- Autonomic dysfunction can impair exercise tolerance. In a study of individuals with and without CAN, Kahn et al. (35) showed a reduced response in heart rate and blood pressure during exercise in individuals with

CAN. Roy et al. (36) demonstrated a decreased cardiac output in response to exercise in individuals with CAN. The severity of CAN has also been shown to correlate inversely with an increase in heart rate at any time during exercise and with the maximal increase in heart rate. It should also be noted that decreased ejection fraction, systolic dysfunction, and diastolic filling limit exercise tolerance (1). Given the potential for impaired exercise tolerance, it has been suggested that diabetic patients who are likely to have CAN have cardiac stress testing before undertaking an exercise program.

Orthostatic hypotension-Orthostatic hypotension is defined as a fall in blood pressure (i.e., >20 mmHg for systolic or >10 mmHg for diastolic blood pressure) in response to postural change, from supine to standing (37). In patients with diabetes, orthostatic hypotension is usually due to damage to the efferent sympathetic vasomotor fibers, particularly in the splanchnic vasculature. In addition, there is a decrease in cutaneous, splanchnic, and total vascular resistance that occurs in the pathogenesis of this disorder.

Normally, in response to postural change there is an increase in plasma norepinephrine. For individuals with orthostatic hypotension, there may be a reduction in this response relative to the fall in blood pressure (38). Diminished cardiac acceleration and cardiac output, particularly in association with exercise, may also be important in the presentation of this disorder (38). Less frequently, there is a rise in norepinephrine that may be due to low blood volume or reduced red cell mass. Frequently, there are fluctuations in the degree of orthostatic hypotension. This may reflect postprandial blood pooling, the hypotensive role of insulin, and changing patterns of fluid retention due to renal failure or congestive heart failure . Cardiovascular autonomic function testing may help differentiate CAN from other causes of weakness, lightheadedness, dizziness, or fatigue and promote appropriate therapeutic intervention.

Silent myocardial ischemia/cardiac denervation syndrome.

A reduced appreciation for ischemic pain can impair timely recognition of myocardial ischemia or infarction and thereby delay appropriate therapy.

OTHER AUTONOMIC NEUROPATHIES

Assessing GI autonomic function.

Gastroparesis should be suspected in patients with erratic glucose control. The finding of retained food in the stomach after an 8- to 12-h fast in the absence of obstruction is diagnostic of gastroparesis. Basic diagnostic tests include upper-GI endoscopy or barium series to rule out structural or mucosal abnormalities of the GI tract. Evaluation of the patient with suspected diabetic gastroparesis might include the following: Assessment of glycemic control,Medication history, including the use of anticholinergic agents, ganglion blockers, and psychotropic drugs, Gastroduodenoscopy to exclude pyloric or other mechanical obstruction, Manometry to detect antral hypomotility and/or pylorospasm, Double-isotope scintigraphy to measure solid-phase gastric emptying; this requires ingestion of a solid labeled with radionuclides. Liquid emptying gives false-negative results. The blood glucose should be normal at the time of testing because hyperglycemia decreases gastric motility. Electrogastrography detects abnormalities in GI pacemaking, but its role has not been established in diagnosis or treatment decision making.

Most of the specialized evaluations for assessment of gastroparesis, constipation, diarrhoea will typically be performed by a gastroenterologist.

Genitourinary autonomic neuropathy

The neurogenic bladder, also called cystopathy, may be due to DAN. The earliest bladder autonomic dysfunctions are sensory abnormalities that result in impaired bladder sensation, an elevated threshold for initiating the micturition reflex and an asymptomatic increase in bladder capacity and retention. When there is damage to the efferent parasympathetic fibers to the

urinary bladder, symptoms such as hesitancy in micturition, weak stream, and dribbling ensue, with a reduction in detrusor activity (i.e., detrusor areflexia). This leads to incomplete bladder emptying, an increased postvoid residual, decreased peak urinary flow rate, bladder overdistention, and urine retention. Finally, overflow incontinence occurs because of denervation of the external and internal sphincter (39). Urinary frequency is another commonly associated symptom of autonomic dysfunction of the genitourinary system. Unfortunately, 37–50% of individuals with diabetes have symptoms of bladder dysfunction, and 43–87% of individuals with type 1 diabetes have physiological evidence of bladder dysfunction (39). Specialized assessment of bladder dysfunction will typically be performed by a urologist.

Erectile dysfunction & Sexual dysfunction in women

Erectile dysfunction (ED) is the most common form of organic sexual dysfunction in males with diabetes, with an incidence estimated to be between 35 and 75% (40). ED is defined as the consistent inability to attain and maintain an erection adequate for sexual intercourse, usually qualified by being present for several months and occurring at least half the time. ED is a marker for the development of generalized vascular disease and for premature demise from a myocardial infarct, and penile failure may be a portent of upcoming, and possible preventable, cardiovascular events. ED etiology in diabetes is multifactorial, including neuropathy, vascular disease, metabolic control, nutrition, endocrine disorders, psychogenic factors, and anti-diabetes drugs. Females with diabetes may have decreased sexual desire and increased pain during intercourse and are at risk of decreased sexual arousal and inadequate lubrication .

Hypoglycemia-induced autonomic failure

The spectrum of reduced counterregulatory hormone responses (in particular epinephrine) and decreased symptom perception of hypoglycemia due to decreased ANS activation after recent antecedent hypoglycemia has been termed "hypoglycemia-induced autonomic failure" (41). Hypoglycemia-induced autonomic failure leads to a vicious cycle of hypoglycemia unawareness that

induces a further decrease in counterregulatory hormone responses to hypoglycemia. This vicious cycle occurs commonly in individuals with diabetes who are in strict glycemic control. The reduced epinephrine response to antecedent hypoglycemia occurs in the absence of DAN as measured by standard tests of autonomic function (42). The presence of autonomic neuropathy, however, further attenuates the epinephrine response to hypoglycemia in diabetic subjects after recent hypoglycemic exposure in most, but not all, studies (42). Furthermore, individuals with abnormal autonomic function have a greater risk for severe hypoglycemia .

Peripheral neurovascular responses.

Smooth muscle microvasculature in the periphery reacts sympathetically to a number of stressor tasks. These may be divided into those dependent on the integrity of the central nervous system (orienting response and mental arithmetic) and those dependent on the distal sympathetic axon (handgrip and cold pressor tests): **Orienting response.** Orienting response is the vasoconstriction and resulting drop in peripheral (index finger, pulp surface) skin blood flow when a subject engages in speech after several minutes of relaxation with music. **Mental arithmetic.** Mental arithmetic as a serial subtraction task typically results in a 30% reduction in peripheral (index finger, pulp surface) skin blood flow. There is no response in the presence of either a proximal or distal ANS lesion **Hand grip.** Peripheral contralateral (index finger, pulp surface) response to sustained 40% maximum grip on a dynamometer is biphasic over 60 s. The initial normal response is 40–50% reduction of flow from basal during the initial 20–30 s, followed by a dilation resulting in a return to typically super-basal levels; there is no response if the peripheral ANS is damaged. **Cold pressor.** Immersion of the contralateral hand in cold (ice) water typically results in a 50–60% reduction in peripheral skin blood flow at the contralateral pulp index surface. In some individuals, this response becomes biphasic after prolonged exposure (30 s) to such intense cold because it is extremely uncomfortable. There is a predominately peripheral component, but pain generates a centrally mediated response.

Assessing pupillary function

Patients with DAN show delayed or absent reflex response to light and diminished hippus due to decreased sympathetic activity and reduced resting pupillary diameter (6). Pupillary measurements are usually only performed in a research setting.

Autonomic Function Tests Assessed In The Current Study

Heart rate response to deep breathing (i.e.beat-to-beat heart rate variation)

Beat-to-beat variation in heart rate with respiration depends on parasympathetic innervation. Pharmacological blockade of the vagus nerve with atropine all but abolishes respiratory sinus arrhythmia, whereas sympathetic blockade with the use or pretreatment of propranolol has only a slight effect on it. Several different techniques have been described in clinical literature, but measurement during **paced deep breathing is considered the most reliable**. The patient lies quietly and breathes deeply at a rate of six breaths per minute (a rate that produces maximum variation in heart rate) while a heart monitor records the difference between the maximum and minimum heart rates. Over a number of years, there have been several different measures of R-R variation. The following six measures have most consistently been reported (standard deviation, coefficient of variation, mean circular resultant, maximum minus minimum, expiration-to-inspiration [E:I] ratio, and spectral analysis) (33)

Heart rate response to standing.

This test evaluates the cardiovascular response elicited by a change from a horizontal to a vertical position. The typical heart rate response to standing is largely attenuated by a parasympathetic blockade achieved with atropine . In healthy subjects, there is a characteristic and rapid increase in heart rate in response to standing that is maximal at approximately the 15th beat after standing. This is followed by a relative bradycardia that is maximal at approximately the 30th beat after standing. In patients with diabetes and autonomic neuropathy, there is only a gradual increase in heart rate. The patient is connected to an electrocardiogram (ECG) monitor while lying down and then stands to a full upright position. ECG tracings are used to determine the 30:15

ratio, calculated as the ratio of the longest R-R interval (found at about beat 30) to the shortest R-R interval (found at about beat 15). Because the maximum and minimum R-R intervals may not always occur at exactly the 15th or 30th beats after standing, Ziegler et al. (43) redefined the maximum/minimum 30:15 ratio as the longest R-R interval during beats 20–40 divided by the shortest R-R interval during beats 5–25.

Valsalva maneuver.

In healthy subjects, the reflex response to the Valsalva maneuver includes tachycardia and peripheral vasoconstriction during strain, followed by an overshoot in blood pressure and bradycardia after release of strain. The response is mediated through alternating activation of parasympathetic and sympathetic nerve fibers. Pharmacological blockade studies using atropine, phentolamine (an alpha-adrenergic antagonist), and propranolol (a nonspecific ß-adrenergic blocker) confirm dual involvement of autonomic nerve branches for the response to this maneuver by demonstrating the drugs' varied effects of attenuation or augmentation of the hemodynamic response to the maneuver at specific times during the response. In patients with autonomic damage from diabetes, the reflex pathways are damaged. This is seen as a blunted heart rate response and sometimes as a lower-than-normal decline in blood pressure during strain, followed by a slow recovery after release.

In the standard Valsalva maneuver, the supine patient, connected to an ECG monitor, forcibly exhales for 15 s against a fixed resistance (40 mmHg) with an open glottis. A sudden transient increase in intrathoracic and intra-abdominal pressures, with a consequent hemodynamic response, results. With performance of the Valsalva maneuver, there is a transient increase in intraocular and intracranial pressure, creating a small theoretical risk of intraocular hemorrhage and lens dislocation (44). In practical terms, however, the risk is minimal because comparable pressures occur in the performance of daily activities. The response to performance of the Valsalva maneuver has four phases and in healthy individuals can be observed as follows: **Phase I:** Transient

rise in blood pressure and a fall in heart rate due to compression of the aorta and propulsion of blood into the peripheral circulation. Hemodynamic changes are mostly secondary to mechanical factors. **Phase II:** Early fall in blood pressure with a subsequent recovery of blood pressure later in the phase. The blood pressure changes are accompanied by an increase in heart rate. There is a fall in cardiac output due to impaired venous return causing compensatory cardiac acceleration, increased muscle sympathetic activity, and peripheral resistance. **Phase III:** Blood pressure falls and heart rate increases with cessation of expiration. **Phase IV:** Blood pressure increases above the baseline value (overshoot) because of residual vasoconstriction and restored normal venous return and cardiac output.

The Valsalva ratio is determined from the ECG tracings by calculating the ratio of the longest R-R interval after the maneuver (reflecting the bradycardic response to blood pressure overshoot) to the shortest R-R interval during or shortly after the maneuver (reflecting tachycardia as a result of strain). With regard to the progression of autonomic dysfunction in diabetes, the Valsalva maneuver may be the best method to monitor this longitudinally (45). Quantitative analysis of nerve function (e.g. autonomic function testing) parallels that of clinical neuropathy in that the rate of progression is slow, gradual, and an insidious process. In a study by Levitt et al. (45), the rate of deterioration of the Valsalva ratio was 0.015 per year for individuals with type 1 diabetes, which was more than twice that expected from cross-sectional studies of the aging effect in normal individuals of a similar age range. All of the tests described above for the assessment of cardiovascular autonomic function can be performed by a general practitioner. Those patients with cardiovascular autonomic dysfunction who have system-specific symptoms will need to be referred to a specialist for refined testing.

Assessing cardiovascular adrenergic (sympathetic) function

Systolic blood pressure response to standing.

Blood pressure normally changes only slightly on standing from a sitting or supine position. The response to standing is mediated by sympathetic nerve

fibers. In healthy subjects, there is an immediate pooling of blood in the dependent circulation resulting in a fall in blood pressure that is rapidly corrected by baroreflex-mediated peripheral vasoconstriction and tachycardia. In normal individuals, the systolic blood pressure falls by <10 mmHg in 30 s. In diabetic patients with autonomic neuropathy, baroreflex compensation is impaired. A task force of the American Academy of Neurology (AAN) and the American Autonomic Society defined orthostatic hypotension as a fall in systolic blood pressure of 20 mmHg or diastolic blood pressure of 10 mmHg accompanied by symptoms (37).

Summary of the mechanism behind autonomic test

Heart rate response to deep breathing is for the most part a function of parasympathetic activity, although the sympathetic nervous system may affect this measure . Similarly, it is parasympathetic activity that plays the greatest role in the heart rate regulation for short-term standing, where the act of standing involves low-level exercise and parasympathetic tone is withdrawn to produce a sudden tachycardic response. In response to subsequent underlying blood pressure changes while standing, a baroreceptor-mediated reflex involves the sympathetic nerves for further heart rate control. Heart rate response to the Valsalva maneuver is influenced by both parasympathetic and sympathetic activity. Measurements of blood pressure response to standing is used to assess sympathetic activity.

CURRENT GUIDELINES FOR THE DIAGNOSIS OF AUTONOMIC NEUROPATHY

Two meetings (the San Antonio Conference on Diabetic Neuropathy held in 1988 and a second conference in 1992) jointly sponsored by the American Diabetes Association and AAN recommended three tests that would facilitate the comparison of results from one clinical investigation to another. The three tests were heart rate response to *1*) deep breathing, *2*) standing, and *3*) the Valsalva maneuver. Two tests of blood pressure control were also recommended: blood pressure response to *1*) standing or passive tilting and *2*) sustained handgrip. These tests were judged suitable for both routine screening and monitoring the progress of autonomic neuropathy (46). No tests of sweating, sympathetic skin

responses, pupillary reflexes, or genitourinary or GI function were considered to be sufficiently well standardized for routine clinical use.

The San Antonio consensus panel further extended the utility of tests of cardiovascular autonomic function by suggesting that a battery of tests could be used to stage patients with autonomic neuropathy and to follow the progression of autonomic function over time (46). A three-stage model was proposed as follows: Early stage: abnormality of heart rate response during deep breathing alone. Intermediate stage: an abnormality of Valsalva response. Severe stage: the presence of postural hypotension.

The San Antonio Consensus Panel also made several general recommendations regarding the need to fully classify DAN:
1) Noninvasive validated measures of autonomic neural reflexes should be used as specific markers of autonomic neuropathy if end-organ failure is carefully ruled out and other important factors such as concomitant illness, drug use, and age are taken into account.

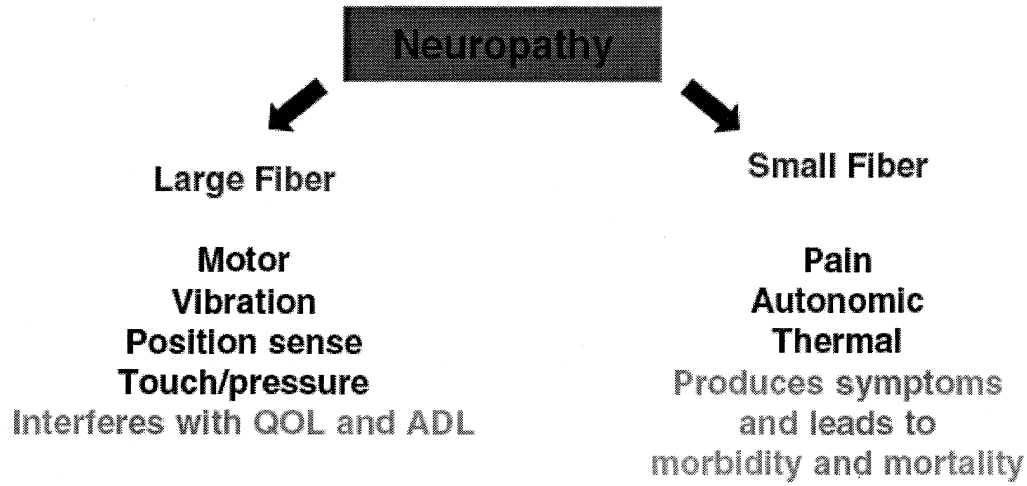
2) An abnormality on more than one test on more than one occasion is desirable to establish the presence of autonomic dysfunction.

3) Independent tests of both parasympathetic and sympathetic function should be performed.

The panel in 1992 also revised its recommendation to include three tests for the longitudinal testing of the cardiovascular ANS: *1*) heart rate response during deep breathing, *2*) Valsalva maneuver, and *3*) postural blood pressure testing (47).

Diabetic peripheral neuropathy.

The most prevalent clinical diabetic neuropathic syndrome is a distal symmetric primarily sensory polyneuropathy with or without accompanying autonomic neuropathy. This syndrome is uncommon early in insulin-dependent diabetes but may be present at time of diagnosis of noninsulin- dependent diabetes.



Clinical presentation of the large- and small-fiber neuropathies.

Less common neuropathic syndromes associated with diabetes should be distinguished from diabetic polyneuropathy. They should be recognized and excluded in studies of the prevalent syndrome described above. Although these unusual syndromes may provide instructive information relevant to polyneuropathy, they probably have varied pathogeneses, natural histories, and responses to therapy. These syndromes include acute focal neuropathies involving the spinal roots, plexuses, and peripheral and cranial nerves. Acute painful neuropathic syndromes that may be associated with the institution of improved metabolic control should also be excluded from clinical trials of typical diabetic polyneuropathy, because their natural history may be different. Late complications of distal symmetric polyneuropathy include various foot ulcerations and deformities, which could be assessed in specific clinical studies addressing these particular late sequelae.

SAFETY OF TESTING PROCEDURES

An expert panel from the AAN reviewed a number of standardized measures and found that noninvasive autonomic tests were found to have a high value-to-risk ratio (48). Some tests do, however, carry a small risk for an adverse event. The Valsalva maneuver transiently increases intrathoracic, intraocular, and intracranial pressure, creating, for example, a small theoretical risk of intraocular

hemorrhage and lens dislocation (48). In practical terms, the risk is minimal because comparable intrathoracic pressures occur in the performance of daily activities. In the published literature of over 100 studies, there have been no reports of deaths during testing and no reports of adverse events after completion of the tests attributable to the procedures. The Diabetes Control and Complications Trial (DCCT), one of the largest trials to use cardiovascular autonomic function tests, evaluated 1,441 patients with type 1 diabetes in 29 centers over a mean duration of 6.5 years without procedural complications (49). When used by properly trained individuals, autonomic function tests are a safe and effective diagnostic tool.

Patient cooperation is required for performing autonomic function tests. Thus, children may pose some challenges related to performance (such as the attainment of the expiration pressure target required for the Valsalva maneuver and the performance of metronomic breathing) and the cooperation and attention requirements of the test situation. These same challenges may also apply to elderly patients, where deterioration of physiological response is of concern, and to developmentally and cognitively disabled individuals.

Although there is an association between the presence of peripheral somatic neuropathy and DAN, researchers have reported that the appearance of parasympathetic dysfunction may be independent of peripheral neuropathy (50). Weinberg and Pfeifer (51) have also shown that reduced HRV may be predictive of the development of symptomatic somatic neuropathy, although these results require follow-up in a larger study cohort. Therefore, assessment modalities that are used to measure other forms of diabetic peripheral neuropathy, such as tests of sensory or motor nerve fiber function (e.g., monofilament probe, quantitative sensory tests, or nerve conduction studies) and tests of muscle strength, may not be effective in detecting the cardiovascular involvement that autonomic function tests detect at early stages of emergence. Thus, tests for other forms of diabetic peripheral neuropathy should not be substituted for tests of cardiovascular autonomic dysfunction.

OBJECTIVES OF THE STUDY

- Evaluate patients with diabetic peripheral neuropathy and to assess evidence of autonomic dysfunction by means of simple, bedside tests.
- Classify patients with diabetic peripheral neuropathy and to no, mild, moderate, severe autonomic neuropathy based on the classification proposed by Ewing et al.
- Correlation between autonomic neuropathy with diabetes disease duration, glycemic control and stage of diabetic peripheral neuropathy.
- Correlation between autonomic neuropathy with other end organ damage due to diabetes mellitus (both micro& macro vascular) especially with coronary artery disease.

MATERIALS AND METHODS

Setting

Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, is a tertiary care center for Neurology in South India. The electrophysiology lab where nerve conduction and electromyographic studies are done caters to around 800-900 patients a year.124 patients were recruited for the study from a total of around 2000 patients evaluated for neuropathy between during January 2006 – June 2008.

Design

Prospective descriptive study, enrolling these patients, with detailed Neurological Symptom Score (NSS)Evaluation, Neurologic Disability Score (NDS)assessment, Nerve conduction Studies, EMG and autonomic function tests . Study was done during January 2006 – June 2008.

Criteria for the diagnosis of diabetes were

1. Symptoms of diabetes and a random plasma glucose $\geq$ 200 mg/dl (11.1 mmol/l). Random is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss.OR
2. FPG $\geq$ 126 mg/dl (7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 h.OR
3. 2-h plasma glucose $\geq$200 mg/dl (11.1 mmol/l) during an OGTT. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75-g anhydrous glucose dissolved in water.

115 patients with diabetic peripheral neuropathy were enrolled into the study. Autonomic function tests were also done on a further 9 diabetic patients who were referred to rule out carpel tunnel syndrome but did not have any evidence for peripheral neuropathy

CLINICAL TESTING OF AUTONOMIC FUNCTION

Assessing cardiovascular autonomic function

Ewing et al. (52) proposed five simple noninvasive cardiovascular reflex tests (i.e., Valsalva maneuver, heart rate response to deep breathing, heart rate response to standing up, blood pressure response to standing up, and blood pressure response to sustained handgrip) that have been applied successfully by many. Of these all except blood pressure response to sustained handgrip are well standardized with age related normative data. Therefore only those 4 tests were used in addition to SSR as tests for autonomic dysfunction.

STANDARDIZED TESTS OF AUTONOMIC FUNCTION

To reduce great variability in assessing the autonomic nervous system, it is important to standardize the test where possible. It is known that eating, drinking coffee, smoking, volume status, upright posture, medicines, and exercise may affect the cardiovascular autonomic nervous system and, presumably, other autonomic nervous organ systems. Therefore, in an ideal situation, studies should be performed with the patient having had no acute illness for the preceding 48 h; unaccustomed vigorous exercise for 24 h, anticholinergic drugs (including antidepressants), antihistamines and over-thecounter cough and cold medications, 9-cu-fluorohydrocortisone, diuretics, sympathomimetic and parasympathomimetic medications,and aspirin for 18 h; alcohol or hypoglycemic episodes for 12 h; or food, caffeine, or tobacco products for 8 h. Moreover, the studies should be performed in the morning in a quiet relaxed atmosphere. The patient should have been taught and have practiced the procedure, and at the time of the study should not be wearing compressive clothing or Jobst stockings, should have the blood glucose stabilized (with insulin, if necessary), and should not be emotionally upset.

The important criteria for appraising clinical tests of autonomic function include reliability, reproducibility, general correlation with each other and with tests of peripheral somatic nerve function, well-established normal values, and demonstrated prognostic value. Three tests of cardiovascular autonomic

nerve function that fulfill these criteria are *1*) the E:I ratio (obtained from R-R variations), *2*) the Valsalva ratio, and *3*) the standing 30:15 ratio. These tests use deep breathing, the Valsalva maneuver, and standing from a supine position, respectively, as provocative stimuli. At least two of these three tests should be performed to provide adequate diagnostic information. An abnormal result for each test is defined as HRV below that of the 5th percentile of the normal age-matched population (as described by Phillipe and Lowe was used). Abnormal HRV in one test is indicative of early autonomic neuropathy.

The sensitivity, specificity, and positive/negative predictive values listed in table 2 summarize results obtained using standardized algorithms and an off site processing center. These currently unpublished data (from A.I.V. and Risk) were based on standardized testing of 205 normal subjects and 3,516 patients with type 1 or type 2 diabetes from 42 centers.

Table 2— Summary of HRV test performance (From A.I. Vinik and M.Risk).

	E:I ratio	Valsalva ratio	Standing (30:15) ratio
Sensitivity	0.93	0.98	0.93
Specificity	0.93	0.91	0.93
Positive predictive value	0.93	0.91	0.92
Negative predictive value	0.94	0.98	0.93

Ewing et al. (52) used (1) HRV during deep breathing (2)Valsalva maneuver(3) 30:15 ratio(4)BP response to standing(5)BP response to handgrip based on which he classified diabetic autonomic neuropathy as follows.

Normal (no autonomic neuropathy) = all tests normal.

Early (mild) = one of the three heart rate tests abnormal.

Definite (moderate) = two or more of the heart rate tests abnormal;

Severe = at least two of the heart rate tests abnormal and one or both of the BP tests abnormal.

E:I ratio

The beat-to-beat HRV assesses the heart rate response to an autonomic reflex arc using an electrocardiograph and a means for standardizing the patient's breathing rate (e.g., visual cues to guide inspiration and expiration). The time intervals between R-waves of the QRS complexes are measured in milliseconds. This measurement was obtained using the deep respiration test and the results evaluated by determining the E:I ratio.

To perform the test, the subject remains supine and breathes deeply at the rate of one breath per 10 s (i.e., six breaths per minute) for 1 min while being monitored by ECG. The E:I is the ratio of the mean of the longest R-R intervals during deep expirations to the mean of the shortest R-R intervals during deep inspirations. Shifting of the heart rate and regularity of the respiratory cycling significantly affect the E:I ratio. HRV decreases with increasing respiration rate, with the greatest variation occurring at a respiratory rate of six breaths per minute. Respiration was therefore standardized at six breaths per minute to optimize test results. E:I ratios are based on the fact that inspiration shortens R-R intervals while expiration lengthens them.

R-R variation is influenced by many physiological factors:

a). Respiratory rate. There is a decrease in R-R variation with increasing respiration rate. The greatest R-R variation occurs at a respiratory rate of ***5*** breathdmin. Thus, it isnot only important to standardize the respiration rate, but the rate should be 5 breaths/min to optimize the results.

b). Age. R-R variation decreases with age and allowance must be made for this.

c). Weight. Parasympathetic activity is reduced in obese individuals.

d). Blood pressure/body temperature. These affect the response in predictable manners based on the heart-rate response.

e). Heart rate. In normal individuals, heart rate increases and R-R variation decreases in a predictable manner with aging.7 In diabetic individuals, changes in heart rate are more complicated. With increasing duration of diabetes there is initially an increase followed by a slowing and finally a fixed heart rate.8 R-R variation, in contrast, decreases early and rapidly after the diagnosis of diabetes has been established.

f). Position. Both standing and sitting significantly reduce RR variation; therefore tests must be done in the lying position.

Confounding variables in R-R autonomic testing':

a. General: this includes eating, drinking coffee, and smoking.

b. Sodium salicylate raises the R-R variation.

c. Dehydration can alter the response.

d.Coronary artery disease: it has been shown that patients with inferior wall myocardial infarctions experience a bradycardia-hypotensive syndrome, whereas patients with anterior wall myocardial infarctions experience tachycardia-hypertensive syndromes. Both can affect the R-R variation.

Valsalva maneuver- In the standard Valsalva maneuver, the supine patient, connected to an ECG monitor, forcibly exhales for 15 s against a fixed resistance (sphygmomanometer used) with an open glottis. The patient should maintain constant pressure at 40 ml over the 15-s interval. This causes a sudden transient increase in intrathoracic and intra-abdominal pressure and a consequent hemodynamic response. The Valsalva ratio is the longest R-R divided by the shortest R-R occurring within 45 s of peak heart rate and is indicative of overall condition of the parasympathetic and sympathetic fibers.

Heart rate response to standing

To test the heart rate response to standing, the patient is connected to the heart rate monitor while in the supine position. The patient then stands to a full upright position, and the ECG is monitored for an additional period while standing. Standing causes an immediate rapid increase in heart rate with the maximum rate generally found at or around the 15th beat after standing. The heart rate slows at or around the 30th beat. The heart rate tracing is used to calculate the ratio of the longest R-R interval (about beat 30) after the stand to the shortest R-R interval (about beat 15). This measure, called the 30:15 ratio, reflects the overall condition of the parasympathetic fibers. Normal ranges are age dependent.

Assessing sudomotor function

Testing of the eccrine sweat glands provides a measure of sympathetic cholinergic function. Thermoregulatory sweat testing assesses both central and peripheral aspects of the efferent sympathetic nervous system, from the hypothalamus to the sweat glands, but is not able to differentiate between pre- and postganglionic causes of anhidrosis. Postganglionic sudomotor function can be determined by measuring sweat output after iontophoresis or intradermal injection of cholinergic agonists. Tests of sudomotor function evaluate the extent, distribution, and location of deficits in sympathetic cholinergic function. These tests include the quantitative sudomotor axon reflex test (QSART), the sweat imprint, the thermoregulatory sweat test (TST), and the sympathetic skin response.

The sympathetic skin response (or peripheral autonomic surface potential) is generated by the sweat glands and overlying epidermis. This response may occur spontaneously or can be evoked by stimuli such as respiration and startle. The sympathetic skin response can be measured with surface electrodes connected to a standard electromyogram instrument. The

response habituates with repeated stimuli and is subject to variability. Delivering stimuli at irregular intervals may minimize habituation.

Diabetic peripheral neuropathy.

Useful explicit clinical definitions for diabetic peripheral neuropathy has been provided by Dyck et al.' They used as the minimal criteria the presence of sensory, motor, or peripheral autonomic signs or symptoms that predominated in the distal segments of the lower extremities, and a Neuropathy Disability Score that was symmetrically abnormal (for the lower limbs it could not vary by **>3** when the Neuropathy Disability Score was <10 [normal ≤2 or by **>25%** when the Neuropathy Disability Score was ≥10). An abnormality on the Neuropathy Disability Score had to be confirmed by an abnormal Neuropathy Symptom Score in one of its subscales (weakness, sensory, or autonomic subscales) or by nerve conduction, or autonomic nervous system tests.

Exclusion of nondiabetic neuropathies

Systematic questioning, including family history of nondiabetic peripheral nerve disease and the presence of toxic, metabolic, mechanical, and vascular causes of nerve disease, was conducted. If any other potentially neuropathic factors are present, other diagnostic methods were used to determine the etiology of nerve disease. Some of the disorders which were considered include nutritional deficiencies, collagen vascular disease, malignancies, tabes dorsalis, toxin exposure (e.g., alcohol, occupational toxins, vitamin B6, and medications known to be associated with peripheral neuropathy [Dilantin therapy for decades, nitrofurantoin, amiodarone, metronidazole, vincristine, cisplatinum, taxol]), hypothyroidism, pernicious anemia, dysproteinemias, amyloidosis, AIDS, chronic idiopathic demyelinating neuropathy, spinal cord disease, cauda equina syndrome, and other mechanical conditions that damage peripheral nerve. Appropriate laboratory screening for these disorders were performed where indicated.

Clinical measures in patient selection.

Since there are several types of diabetic neuropathy, every effort was made to differentiate them. The classification recommended by Dyck et al is a useful approach (53). Several studies show that acute painful neuropathy occurs early in the course of diabetes and has a different clinical course compared with the chronic sensory variety. In assessing the severity of symptoms and the degree of functional impairment, the system of Dyck et al(53) gives a rational basis for clinical purposes. A recent report by this group suggests that when performed by expert examiners, the reproducibility of the Neuropathy Symptoms Score, and Neuropathy Disability Score was acceptably high.

Standardization of examining methods.

The measures mentioned previously (Neuropathy Symptom Score and Neuropathy Disability Score) have been standardized by administering each questionnaire and physical examination to populations of well-defined normal control subjects and subjects with peripheral nerve disease. History and physical examination were included (refer proforma).

In the sensory examination ambiguous findings were considered negative. The response to each test were considered normal, decreased, or absent. The instruments used were **1)** a disposable pin for pain evaluation, **2)** a cotton tip for light touch, **3)** a 128 Hz tuning fork for vibration sensation, and **4)** finger and toe movements with immobilization of the proximal joint to evaluate joint position. The sites examined included the distal toe and distal finger. The motor system was examined manually for individual muscles with a previously used validated grading system. Mechanical devices to evaluate strength may not add precision because they emphasize groups of muscles and because the condition of the joints and periarticular tissues frequently are abnormal in diabetes. Muscle testing is of limited value in assessing mild diabetic neuropathy. Weakness appears late and usually only involves intrinsic foot muscles and ankle dorsiflexors; more proximal muscles are only involved in more severe cases of

diabetic polyneuropathy. Reflexes were classified as **1)** present and active, **2)** present and hypoactive, and **3)** absent.

Electrodiagnostic Measures-Standardization

An EMG technician and neurologist performed the studies. The Nicolet Viking IV system was used. Equipment fulfilled conventional standards for patient use. Recommended filter settings (approximate values) are 20-3,000 Hz bandpass for sensory studies, 2-10,000 Hz bandpass for motor studies, and 20-10,000 Hz bandpass for needle electromyography. Averaging of sensory responses was used to improve the signal-noise ratio. Supramaximal percutaneous stimulation is used for all nerve conduction studies, except for H reflexes. The use of $\geq$20% greater intensity than maximal was adequate and minimized the chance of inadvertent stimulus spread. Motor median conduction studies were performed by using the standard techniques of supramaximal stimulation. The distal motor nerve latency (DML) was measured with an active electrode placed over the muscle belly and the nerve was stimulated used bipolar stimulation electrodes.

1.Amplitude: The CMAP amplitude is measured from the baseline to the peak of the negative wave (M wave). An initial positive deflection usually indicates an improper recording site and the active electrode was repositioned over the motor point. The SNAP amplitude is measured from the baseline (or peak of the initial positivity, if present) to the peak of the negative wave.

2. Distal latency: Distal motor latency is measured to the onset of the M wave. Distal sensory latency may be measured to the peak of SNAP-negative wave when a standardized distance is used, although this measure is inappropriate for conduction velocity calculation. The SNAP onset latency can be measured to the onset of the initial negative deflection and used to calculate a distal sensory conduction velocity.

3. Distance: Distance is measured between the distal stimulation site (cathode) and the midpoint of the active recording electrode. Distance also is measured between stimulation sites when additional stimulation is performed.

4. Conduction velocity: The velocity of the fastest nerve fibers is calculated with CMAP onset latencies between two stimulation (or recording) sites. The same gain and sweep speed are used for distal and proximal stimulation. At both stimulation sites the CMAP configurations should be similar.

5. F wave latency: The minimal latency is measured to the onset of the earliest F wave after 5-15 antidromic motor nerve stimulations from the distal site. A minimum of 5 acceptable F waves is recorded. The F wave is considered unobtainable if there are no responses with 10 stimulations. F waves were distinguished from A waves which demonstrate no latency or waveform variability.

A sample protocol of nerves for evaluation is shown in Table 3

Nerve	Stimulate	Record	Distance
Median S	Wrist	Digit 2	14 cm
	Antecubital fossa		measure
Ulnar S	Wrist	Digit 5	14 cm
Sural S	Midcalf	Lateral malleolus	14 cm
Median M	Wrist	Abductor pollicis brevis	7 cm measure
	Antecubital fossa		
Median F wave	Wrist	Abductor pollicis brevis	
Ulnar M	Wrist	Abductor digit minimi	7 cm measure
	Below elbow; 1 cm distal to condylar groove*		
Ulnar F wave	Wrist	Abductor digit minimi	
Peroneal M	Anterior ankle	Extensor digitorum brevis	9 cm measure
	Lower edge of fibular head*		
Peroneal F wave	Ankle	Extensor digitorum brevis	
Tibial M	Medial malleolus	Abductor hallucis	9 cm measure
	Popliteal fossa		
Tibial F wave	Ankle	Abductor hallucis	

* To avoid common sites of compression.

Sensitivity, specificity, and reproducibility. No electrodiagnostic results are specific for diabetic polyneuropathy. However, electrodiagnostic evidence of axonal degeneration and substantial conduction slowing in the proper clinical setting is suggestive of diabetic polyneuropathy. A reduced conduction velocity has a high sensitivity7 but a low specificity in detecting diabetic polyneuropathy. A reduced SNAP amplitude (especially the sural) has a high specificity and sensitivity in detecting any sensorimotor polyneuropathy(54). Electrodiagnostic abnormalities documenting subclinical diabetic polyneuropathy are well

established for group comparison. The sensitivity of nerve conduction studies has been demonstrated in diabetic patients without neurological symptoms or signs. In such patients, abnormalities of conduction velocity may be prominent, reflecting a metabolic 'abnormality in diabetic nerves or segmental demyelination and remyelination. Reduced SNAP amplitude is another sensitive finding in subclinical and clinical involvement; reduced CMAP amplitude has lower sensitivity in general, but is probably more specific for disabling polyneuropathy. The sensitivity and specificity of F wave latency are not established. H reflex abnormalities have high sensitivity but low specificity because they are found in so many other disorders, as well as with advanced age alone.Electromyography may reveal partial denervation in intrinsic foot muscles as an early sign of diabetic polyneuropathy. As a sensitive indicator of axonal degeneration, the needle examination may demonstrate the only abnormality in some patients with early diabetic polyneuropathy. Even though amplitude measures have good reproducibility for groups, they may vary substantially in the same individual at even short inter-test intervals. In general, nerve conduction velocities have excellent reproducibility for groups and good reproducibility for individuals. F and H wave latencies are less subject to inter-test variability.

PROFORMA(53)

Neurological Symptom Score

Score 1 point for presence of a symptom.

I.Symptoms of muscle weakness

A.Bulbar
1. Extraocular _____
2. Facial _____
3. Tongue _____
4. Throat _____

B.Limbs
5. Shoulder girdle and upper arm _____
6. Hand _____
7. Glutei and thigh _____
8. Legs _____

II.Sensory disturbances

A.Negative symptoms

9. Difficulty identifying objects in mouth _____
10. Difficulty identifying objects in hands _____
11. Unsteadiness in walking _____

B Positive symptoms

12. "Numbness," "asleep feeling,""prickling," at any site _____
13. Pain -burning, deep aching, tenderness at any location_____

III.Autonomic symptoms

14. Postural fainting _____
15. Impotence in male _____
16. Loss of urinary control _____
17. Night diarrhea _____

Scoring:Weakness is scored:0 = normal, 1= 25%, 2= 50%, 3= 75%, and 4= 100%.Papilledema is scored: 0 = absent, 1 = present. Reflexes and sensation are scored: 0 = normal, -1 = decreased, -2 = absent (53)

Cranial nerves	Right	Left
• Papilliedema	_____	_____
• EOM weakness, Cr III	_____	_____
• EOM weakness, Cr VI	_____	_____
• Face weakness	_____	_____
• Palate weakness	_____	_____
• Tongue weakness	_____	_____
Muscle weakness		
• Respiratory	_____	_____
• Shoulder abduction	_____	_____
• Biceps brachii	_____	_____
• Brachioradialis	_____	_____
• Extension at elbow	_____	_____
• Extension at wrist	_____	_____
• Flexion at wrist	_____	_____
• Extension of fingers	_____	_____
• Flexion of fingers	_____	_____
• Intrinsic hand	_____	_____
• Iliopsoas	_____	_____
• Glutei	_____	_____
• Quadriceps	_____	_____
• Hamstrings	_____	_____
• Dorsiflexors	_____	_____
Plantar flexors	_____	_____
Reflexes		
• Biceps brachii	_____	_____
• Triceps brachii	_____	_____
• Brachioradialis	_____	_____
• Quadriceps femoris	_____	_____
• Triceps surae	_____	_____
Sensation		
Index finger (below base of nail; JP at MCP joint)		
○ Touch-pressure	_____	_____
○ Pricking pain	_____	_____
○ Vibration	_____	_____
○ Joint position (JP)	_____	_____
• Great toe (below base of TP at MTP joint)		
Touch-pressure	_____	_____
Pricking pain	_____	_____
Vibration	_____	_____
Joint position	_____	_____
Sum	_____	_____
Total	_____	_____

Staging of Neuropathy(53)

1. Stage 0 (no peripheral neuropathy) Fewer than two abnormalities among (1) NC; (2) NE (e.g.;neurologic examination or NDS); and (3) NS (e.g., neuropathic symptoms, NSS,) (see definitions in the following section) with at least one abnormality being NC .

2. Stage 1(mild peripheral neuropathy) Two or more abnormalities among (1) NC; (2) NE; and (3) NS.

3. Stage 2 (moderate peripheral neuropathy) Two or more abnormalities among (1) NC; (2) NE; and (3) NS. Neuropathic symptoms are present but are of lesser severity than in stage 3.

4. Stage 3 (disabling/severe peripheral neuropathy) Two or more abnormalities among (1) NC; (2) NE; and (3) NS.Disabling neuropathic symptoms are present.

Symptomatic Neuropathy (Stage 2)

Occurrence of any symptoms among the 12 listed below, judged to be due to diabetic polyneuropathy but not disabling is sufficient to fulfill the criteria for stage 2. Patients with questionable degrees of symptoms are staged as 1.

Motor

1. Symptoms of muscle weakness in acts of daily living.

Sensory- Any of the following symptoms:

1.Absence of feeling: reported deficiency of tactile, thermal, or nociceptive sensation encountered in acts of daily living.

2.Sensory ataxia: reported unsteadiness in walking.

3.Numbness or paresthesia judged by its distribution, persistence, and duration to be due to neuropathy and not due to physiologic compression as occurs in acts of daily living, to entrapment or to another condition.

4.Neuropathic pain: burning, aching, excessive discomfort of feet or hands with use, and lancinating pain, considered to be due to diabetic neuropathy.

Autonomic- Any of the following symptoms attributed to diabetes mellitus and not to medications, psychologic disturbance, intercurrent illness, disease of the organ, or previous injury or surgery:

1.Gastric atony

2.Urinary retention

3.Urinary incontinence

4.Rectal incontinence

5.Diarrhea

6.Impotance in males less than 65 years of age.

7.Postural hypotension, light-headedness or fainting

Disabling Neuropathy (Stage 3).

Occurrence of any of the 10 conditions listed below, judged to be due to diabetic neuropathy, results in the diagnosis of the neuropathy as stage 3.

Motor- Symptoms of muscle weakness, confirmed by examination, of sufficient severity that the patient is unable to walk independently.

Sensory

1.Symptoms of sensory loss of sufficient severity, confirmed by examination, that the patient cannot walk independently because of sensory ataxia.

2.Absence of feeling in hands so that the patient is disabled.

3.Symptoms of pain, having the characteristics of neuropathic pain that is disabling. Criteria a, b, and c have to be fulfilled.

a)The patient has previously attended physicians for pain relief.

b)Work and recreational activities have been curtailed by at least 25 per cent because of pain.

c)Medication for pain relief has been taken on a continuing (≥50 per cent of days) basis for at least 6 weeks.

Autonomic

1.Gastric atony. Emesis of retained (≥ 18 h) food at least once weekly for at least 6 weeks.

2.Urinary retention necessitating continuous use of a catheter for 6 weeks or longer.

3.Urinary incontinence necessitating continuous (≥ 50 per cent of time) use of diapers or leg urinal for at least 6 weeks

4.Rectal incontinence due to loss of anal sphincter function of at least 6 weeks

5.Diarrhea to the degree that it causes weight loss (≥5 kg)

6.Symptomatic light-headedness or fainting, present continuously (light-headedness or fainting weekly) for at least 6 weeks.

Patient Data:

Name:

Age/Sex:

Address:

Hospital No/MRD No:

Wt/Ht/BMI:

Type of Diabetes:

Duration of diabetes:

Treatment:

OHA TYPE and duration of treatment.

Insulin TYPE and duration of treatment

FBS/PPBS/HbA1c;

RFT urea/creat;

Other documented diabetic complications:

CAD-nil,EA,UA,MI.post CABG/stent

TIA/CVA-side

nephropathy -microalbuminuria

Other illness (specify).

Duration of symptoms: (from 1st symptom)

A.N.S function tests

1.SSR

2.30:15 HR ratio on standing

3.Orthostatic BP fall

4.HR variation with respiration-RR interval analysis

5.Valsalva maneuver.

Statistical Analysis

All the data were computed and statistical analyses were done using the SPSS PC Windows version 10.0. Students "t" test was used to compare means between groups and Chi square test was used to compare proportions. Fischer's test and ANOVA with bonferroni correction was used where appropriate. Logistic regression analysis was used to assess factors associated with diabetic peripheral and autonomic neuropathy. $p<0.05$ was considered to be statistically significant.

Results of the study:

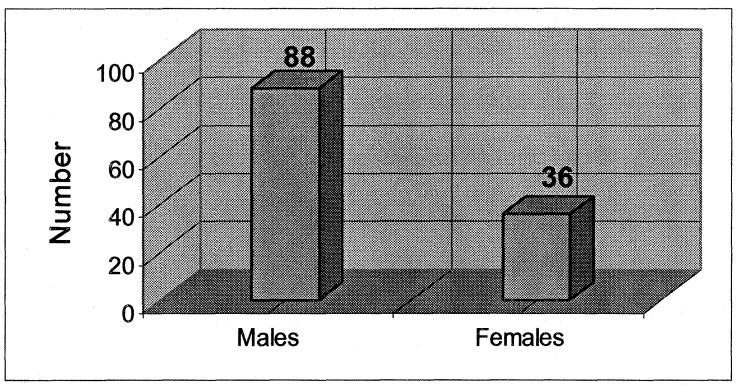
A total of 115 diabetic individuals with peripheral neuropathy who did not have any other alternate cause for the same were selected. In addition the autonomic function tests were also done on 9 other diabetic individuals who did not have peripheral neuropathy but were referred for evaluation of carpal tunnel syndrome (CTS). We had 36 patients with mild, 50 with moderate and 29 with severe peripheral neuropathy. Out of them 35 did not have any evidence for autonomic involvement 40 had mild, 40 moderate and 9 had severe autonomic neuropathy.

The mean age of the study population was 59.62 yrs with a standard error of 0.945 (57.81 – 61.91; 95% CI). Minimum age was 34 and maximum 82 years. There were 71% males (SE = 0.041).

Chart showing sex distribution

	Number	Percent
males	88	71.0
females	36	29.0
Total	124	100.0

Table showing sex distribution



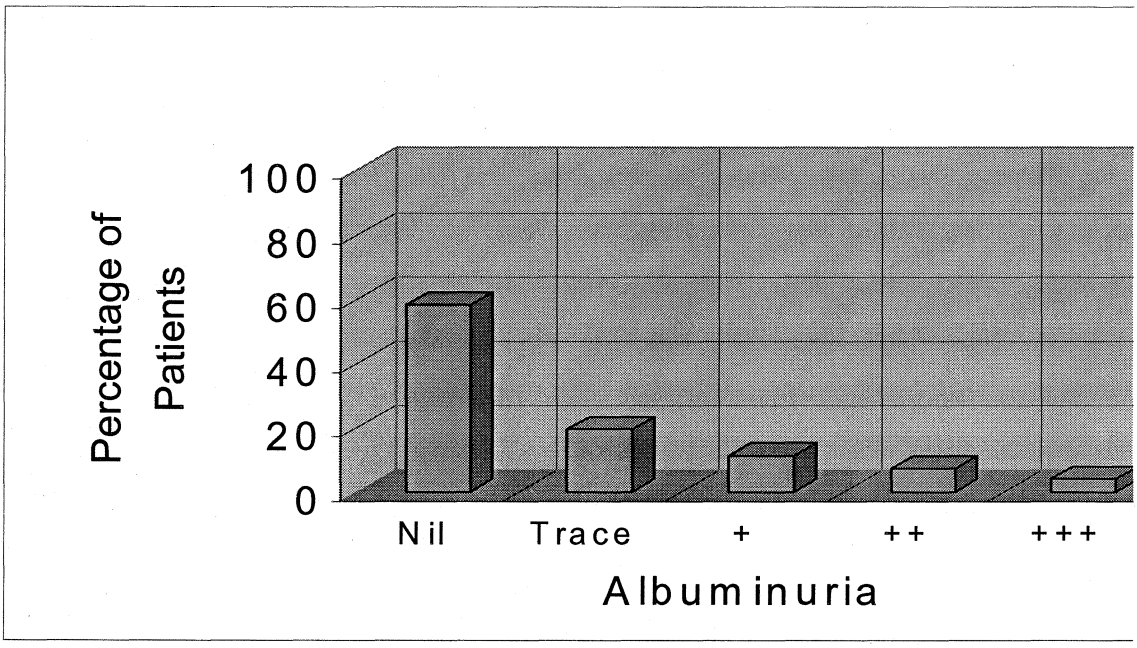
The mean body mass index (BMI) was 25.024 (SE =0.275) (95% CI = 24.472 – 25.576). 61.3% were of optimum BMI (BMI =18 – 24.9) 30.6% were over weight (BMI =25 – 29.9) and 8.1% were obese (BMI $\geq$30). 67.7% had systemic hypertension.

Of the total patient group 2 had maturity onset diabetes of young while the rest had type 2 diabetes mellitus. The mean duration of diabetes was 12.02 years (95% CI = 11.57 – 13.47). Majority (50%) were using a combination of insulin releasers with sensitisers. 37.1% were using insulin either alone or in combination with oral antidiabetic agents. Only 1/3rd of the patient group (33.9%) had good glycemic control as evidenced by a fasting blood sugar (FBS) of less than 100 mg %. 43.5% had FBS between 151 – 200 %. The mean FBS was 125.5 mg % (95% CI = 117.5 – 133.5). The mean postprandial blood sugar (PPBS) was 172.94 mg % (95% CI = 161.46 – 184.42). 36.3% had optimal control of postprandial glycemic status while 9% had very poor control with PPBS values more than > 250mg%. Mean levels of glycosylated haemoglobin level (HbAIc), which is a marker for long term glycemic control was 6.737 gm % (95% CI = 6.54 – 6.93) 40.3% had good control i.e. a HbAIc of < 6.5 gm % while 6.5% had poor control (between 8.6 – 10.5 gm %) while the rest had values in between 6.6 – 8.5gm%. Mean blood urea nitrogen (BUN) levels were 13.41 mg % while mean creatinine was 1.046mgm%. Patients with overt uraemia were avoided from the study.

42% of the patients had evidence for early nephropathy in the form of presence of urine albuminuria, which was graded from trace to 3+. 19.4% had only trace involvement while 4% had 3+ albuminuria while the rest (18.6%) had in between glomerulopathy.

Table showing incidence of albuminuria among patients

albuminuria	Frequency	Percent
Nil	72	58.1
Trace	24	19.4
+	14	11.3
++	9	7.3
+++	5	4.0
Total	124	100.0



The mean total cholesterol was 198.6 mg%. 16.1% had >240 mg% while 57.3% had <200 mg % and the rest in between. 28.2% has had a history of coronay artery disease of which 6.5% had undergone CABG, 2.4% had undergone stent placement while the rest were on pure medical management.

Table showing incidence coronay artery disease(CAD) among patients

CAD	Frequency	Percent
No CAD	89	71.8
medical	24	19.4
Stent	3	2.4
CABG	8	6.5
Total	124	100.0

7.3% patients has had a transient ischemic attack while one patient had an asymptomatic carotid stenosis by doppler evaluation.

50.8% had evidence of diabetic retinopathy. In 35.5% patients it was limited to background retinopathy while the rest (15.3%) had proliferate retinopathy.Of the 115 patients with diabetic peripheral neuropathy out of the total neuropathic motor symptom score of 8, 2 patients had the maximum score of 4(1.7%). 61.7%

with peripheral neuropathy had motor scores of zero while 36.6% has scores either 1 or 2 (SE=0.082).

Out of the total Neuropathy Symptom Score sensory (NSS-S) of 5, 93% patients had atleast a score of ≥1.Of the rest 7% only one patient presented with pure motor symptoms while others had presented with autonomic symptoms or a combination of motor and autonomic symptoms. 61.7% patients had a sensory score of 1, 1.7% (2 patients) had a maximum score of 4 while the rest had scores between 1 and 4 (SE=0.069). Out of total 115 only 34.8% patients had co-existent autonomic symptoms of which 6.1% had a symptom score of 2 (total score = 4) while the rest had a score of 1 (SE=0.056). Of the total NSS of 17, the maximum score obtained was 7 (seen in one patient). About 80% patients had scores of either 1,2 or 3 (SE=0.143).

The mean Neuropathic disability score (NDS) was 19.48 (of a total of 228). NDS scores were subdivided in to motor (mean= 3.7 of a total of 176), sensory (mean of 7.43 of a total of 32) and reflexes (mean of 8.41 of a total of 20). Diminished or absent reflexes were the most common objective finding seen in 85.2% of patients which was closely followed by sensory findings.

Among the autonomic involvement significant postural fall in blood pressure (systolic ≥20 mm of Hg) was seen in 5 patients (4.3%) while 20 had BP fall between 10-19 mm of Hg (20%). Among the tests for heart rate variability valsalva was the most sensitive being abnormal in 61.3% closely followed by expiration: inspiration ratio which was abnormal in 48.4%. 30:15 ratio was the least sensitive among the tests for heart rate variability being abnormal only in 16.9% patients. Sympathetic skin response was abnormal in 12.9%.

7.3% patients had severe autonomic neuropathy while 32.3% had mild and a similar percentage had moderate involvement while 28.2% did not have any autonomic involvement. (SE=0.084).

61% had an axonopathic neuropathy, 22.6% had demyelinating neuropathy while rest had demyelinating with axonopathic features. The motor nerves tested were bilateral tibial, peroneal and ulnar. At least ≥1 inelicitable motor nerves was seen in 17.4% of patients. while 79% had atleast ≥1 abnormal motor nerves.

40% patients had ≥1 inelicitable sensory nerves while 81% had atleast ≥1 abnormal sensory nerves. (SE =0.109). Mean peroneal CMAP was 3.67 mV (SE=0.303) while the mean peroneal distal latency was 4.58 millisecond (SE=0.206).

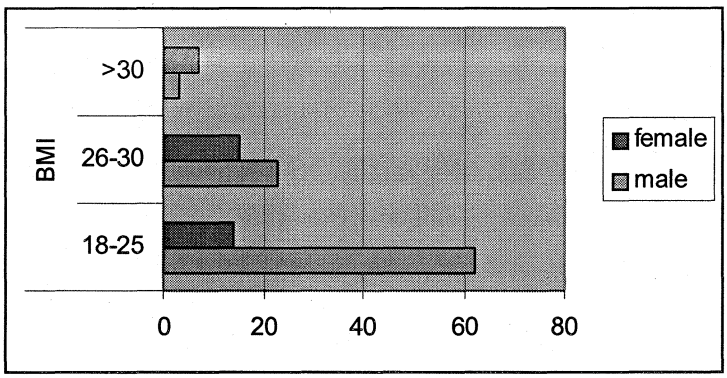
Bivariate analysis of peripheral neuropathy: Chi square test was done to assess the BMI between males and females. Females were more likely to be either over weight or obese than males (P=0.001).

Table showing comparison of BMI among males Vs females

		Sex		
		male	female	Total
BMI	18-25=1	62	14	76
	26-30=2	23	15	38
	>30=3	3	7	10
Total		88	36	124

Chi square test (p = 0.001)

Bar graph showing comparison of BMI among males Vs females



ANOVA with bonferroni correction done to assess the relation between age and severity of diabetic neuropathy did not reveal any statistically significant relation between them (P=0.141). Chi-square used to assess the severity of diabetic neuropathy between sexes did not reveal any significant relationship (P=0.667).

ANOVA with bonferroni correction done to assess the severity of diabetic neuropathy with duration of diabetes showed a statistically significant relationship. (p ≤0.001).Patients with mild neuropathy had mean diabetes duration of 9.17 years, those with moderate 11.60 years and those with severe

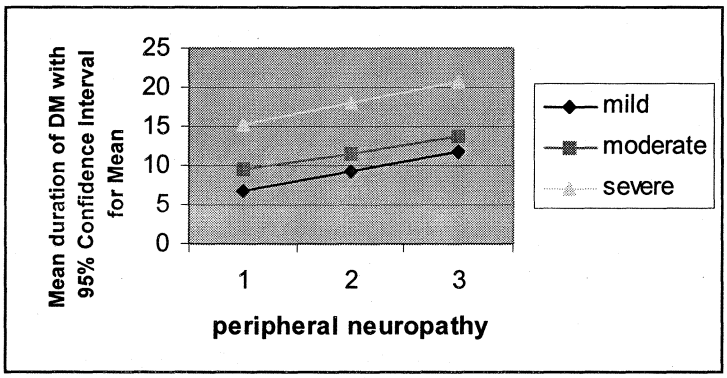
17.98 years (95% CI for mild = 6.7 – 11.64, moderate =9.43 – 13.76, severe= 15.22 – 20.74).

Table showing severity of peripheral neuropathy & duration of DM

peripheral neuropathy	Mean duration of DM	Std. Error	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
mild	9.17	1.216	6.70	11.64
moderate	11.60	1.077	9.43	13.76
severe	17.98	1.347	15.22	20.74
Total	12.45	.756	10.95	13.94

ANOVA with Bonferroni correction ($p < 0.001$)

Graph showing severity of peripheral neuropathy & duration of DM



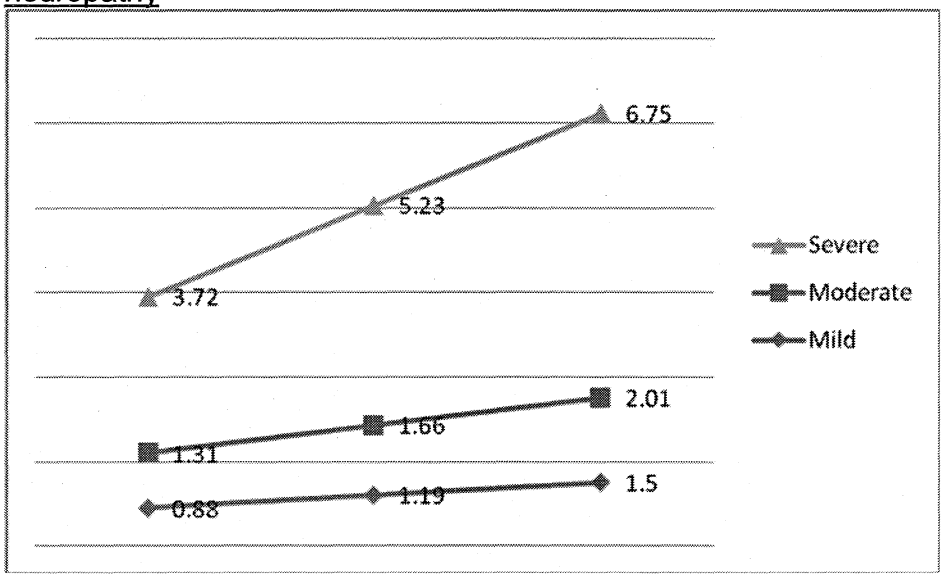
Duration of neuropathic symptoms and severity also had a strong correlation ($P<0.001$). Those with mild had a mean neuropathic symptom duration of 1.19 years (95% CI = 0.88 – 1.50), moderate had 1.66 years (95% CI 1.31 – 2.01) and severe had 5.23 years (95% CI 3.72 – 6.75).

Table showing Duration of symptoms (yrs) Vs severity of diabetic peripheral neuropathy

severity of diabetic peripheral neuropathy	Mean duration of symptoms yrs	Std. Error	95% Confidence Interval for Mean	
		Upper Bound	Lower Bound	Upper Bound
mild	1.19	.154	.88	1.50
moderate	1.66	.174	1.31	2.01
severe	5.23	.741	3.72	6.75
Total	2.41	.256	1.91	2.92

ANOVA with Bonferroni correction ($p < 0.001$)

Graph showing Duration of symptoms (yrs) Vs severity of diabetic peripheral neuropathy



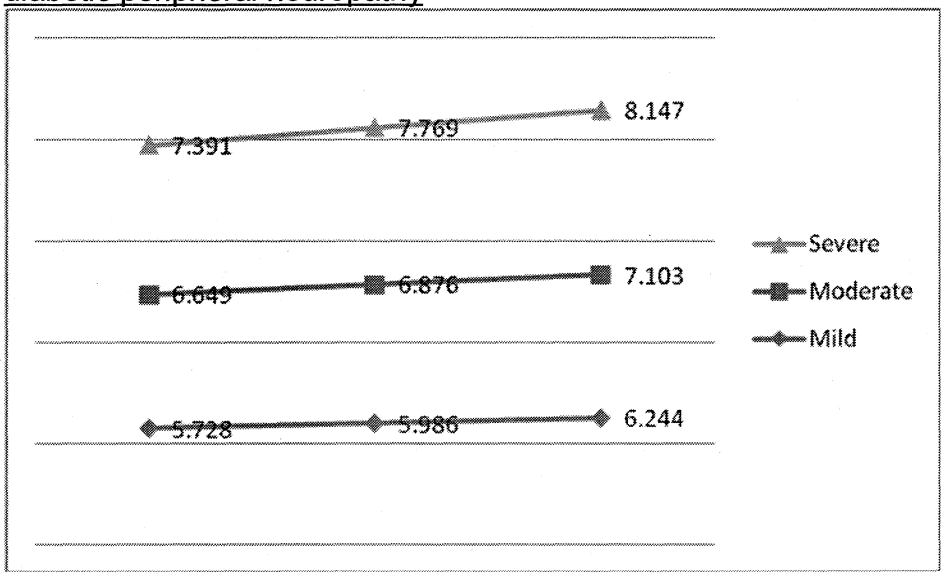
HbA1c levels and severity of diabetic peripheral neuropathy had a strong correlation (P<0.001). Those with mild peripheral neuropathy had a mean HbA1c of 5.986 gm%, moderate had 6.876 gm% and severe had 7.769 gm%.

Table showing HbA1c (gm/dl) Vs severity of diabetic peripheral neuropathy

severity of diabetic peripheral neuropathy	number of patients	Mean HbA1c (gm/dl	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound	Lower Bound	Upper Bound
mild	36	5.986	.7624	.1271	5.728	6.244
moderate	50	6.876	.7994	.1130	6.649	7.103
severe	29	7.769	.9929	.1844	7.391	8.147
Total	115	6.823	1.0702	.0998	6.625	7.020

ANOVA with Bonferroni correction ($p < 0.001$)

Graph showing Mean HbA1c (gm/dl) with 95% Confidence Interval Vs severity of diabetic peripheral neuropathy



The severity of peripheral neuropathy and FBS/PPBS values had a significant corelation p=0.001. The mild peripheral neuropathy group had a FBS/PPBS of 107.6 / 158.4 mgm%, moderate had 131.62 /169.3 mgm% and severe had 144.55/211.17mg%. Pearsons chi square test was used to compare severity of diabetic peripheral neuropathy and albuminuria. 78% of the patients with mild peripheral neuropathy had no albuminuria while 14% with mild disease had only trace albuminuria. 25% of those with severe peripheral neuropathy had either 2+ or 3+ albuminuria. These values were highly significant (p=0.001).

48% of patients with severe peripheral neuropathy had coronary artery disease (CAD) and were on drugs or stent or has undergone CABG while only 30.5% of patients with mild neuropathy had CAD. But these were not statistically significant (P=0.055). 45% of patients with severe peripheral neuropathy had background retinopathy while 64% of those with mild neuropathy did not have any evidence for diabetic retinopathy. Only 30.6% with mild neuropathy had background and 5.6% had proliferative retinopathy. These values were highly statistically significant with P<0.001.Severity of diabetic

peripheral neuropathy and TIA /carotid stenosis by doppler evaluation did not have any significant correlation (p=0.378).

78% of patients with mild peripheral neuropathy did not have any autonomic symptoms while 58.6% of patients with severe peripheral neuropathy had autonomic symptoms (mainly postural fainting and impotance). These were statistically significant, p = 0.016. The severity of diabetic peripheral neuropathy and autonomic neuropathy had a strong correlation (P<0.001). 76% of those with severe peripheral neuropathy had either moderate or severe autonomic neuropathy while among those with mild peripheral neuropathy 33.3% had no autonomic involvement and 44.4% had only mild autonomic neuropathy.

Those with severe peripheral neuropathy had higher pure axonopathic (62%) or axonopathic with demyelinating features (31%) while only 9% were demyelinating compared to those with mild peripheral neuropathy where demyelinating consisted 22.2%. These findings were statistically significant with a p=0.033.The lipid levels and severity of neuropathy did not correlate.

Bivariate analysis of autonomic neuropathy: Bivariate analysis of severity autonomic neuropathy did not reveal any statistically significant correlation with age or BMI (P=0.116 and 0.444 respectively). Duration of neuropathic symptoms had a significant correlation with severity of autonomic neuropathy (p=0.038).

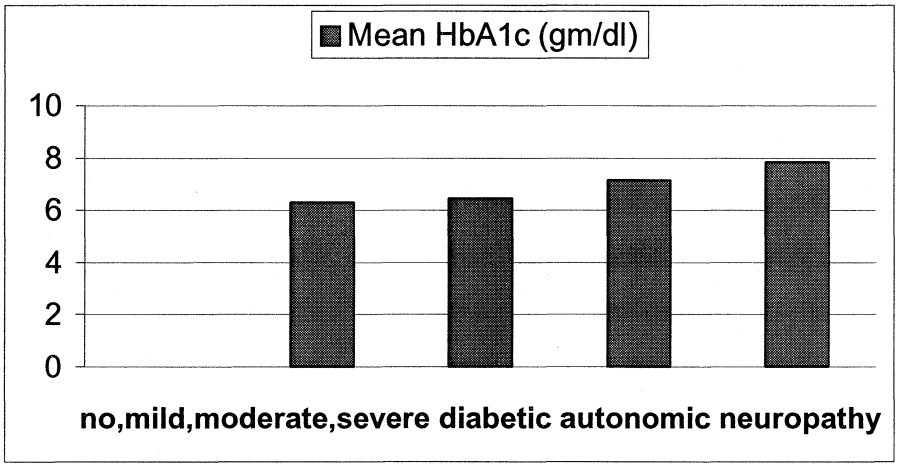
HbA1c had a strong correlation with the severity of diabetic autonomic neuropathy (P<0.001). Those with no autonomic neuropathy had a HbA1c of 6.314 gm% while those with severe had 7.833 gm%.

Table showing HbA1c (gm/dl) Vs severity of diabetic autonomic neuropathy

diabetic autonomic neuropathy	number of patients	Mean HbA1c (gm/dl)	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound	Lower Bound	Upper Bound
no	35	6.314	.8215	.1389	6.032	6.596
mild	40	6.440	.9703	.1534	6.130	6.750
moderate	40	7.158	1.1038	.1745	6.804	7.511
severe	9	7.833	1.0607	.3536	7.018	8.649
Total	124	6.737	1.0814	.0971	6.545	6.929

ANOVA P<0.001

Bar graph showing HbA1c (gm/dl) Vs severity of diabetic autonomic neuropathy



FBS values correlated with severity of autonomic neuropathy but were statistically not significant (p=0.085). But the correlation between PPBS and total cholesterol with autonomic neuropathy were statistically significant (p=0.018 and 0.036 respectively). PPBS of those with no autonomic dysfunction was 148.83 mg% while those with severe autonomic involvement being 203.56 mg%.

1/3 of patients with no autonomic neuropathy did not have albuminuria while 80% of those with 3+ albuminuria had either moderate or severe autonomic neuropathy (p=0.009). Severity of autonomic neuropathy strongly correlated with coronary artery disease (p<0.001) 62.5% of CAD patients on drugs and 100% of those who had undergo CABG had moderate or severe autonomic neuropathy. 74% of patients with no retinopathy had either no autonomic neuropathy or only a mild disease where as 53% of those with proliferate retinopathy had moderate to severe autonomic neuropathy (p=0.003).

Table showing severity of diabetic autonomic neuropathy (no neuropathy ,mild, moderate, severe) Vs CAD(no, medical, stent, CABG)

			CAD				
			No CAD	Medical	stent	CABG	Total
severity of diabetic autonomic neuropathy(1=no neuropathy,2=mild,3=moderate,4=severe)	no	Count	31	2	2	0	35
		%	34.8%	8.3%	66.7%	.0%	28.2%
	mild	Count	33	7	0	0	40
		%	37.1%	29.2%	.0%	.0%	32.3%
	moderate	Count	24	8	1	7	40
		%	27.0%	33.3%	33.3%	87.5%	32.3%
	severe	Count	1	7	0	1	9
		%	1.1%	29.2%	.0%	12.5%	7.3%
	Total	Count	89	24	3	8	124
		%	100.0%	100.0%	100.0%	100.0%	100.0%

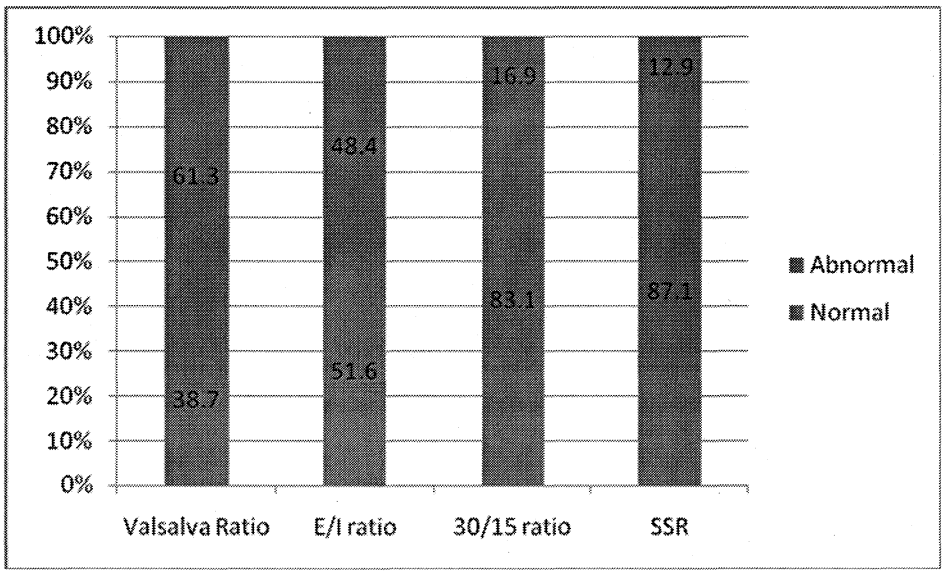
Chi square P <0.001

Among the tests used for autonomic valsalva ratio was the most sensitive which was abnormal in 72.5 % patients followed by E/I ratio.

Table showing autonomic tests and its sensitivity

	Valsalva ratio		E/I ratio		30/15 ratio		SSR (Y/N)	
	Frequency	Percent	Frequency	Percent	Frequency	Percent	Frequency	Percent
normal	48	38.7	64	51.6	103	83.1	108	87.1
abnormal	76	61.3	60	48.4	21	16.9	16	12.9
Total	124	100	124	100	124	100	124	100

Bar graph showing autonomic tests and its sensitivity



MULTIVARIATE ANALYSIS:

Logistic regression analysis of presence autonomic neuropathy was assessed against age, sex, body mass index, severity of peripheral neuropathy, systemic hypertension, duration of diabetes mellitus, duration of symptoms, FBS, PPBS, Hb A1C, urine albumin, coronary artery disease, retinopathy, NSS and NDS. Among them autonomic neuropathy had a strong correlation with PPBS, coronary artery disease, and neuropathy disability score (NDS).

Severe autonomic neuropathy was strongly associated with coronary artery disease, severity of peripheral neuropathy and urine albuminuria.

Logistic regression analysis of *severity of peripheral neuropathy showed relation to duration of symptoms, Hb A1c, and total neuropathic disability score.* Female gender seemed to be protected against peripheral neuropathy but this finding could be due to small sample size.

Logistic regression analysis of the severity of autonomic neuropathy Vs various nerve conduction study parameters revealed that the number of

abnormal motor nerves had the best correlation. Presence of even one abnormal motor nerve increases the risk of mild autonomic neuropathy (p = 0.02) while the presence of 4 or more abnormal/ inelicitable motor nerves increases the risk for severe autonomic neuropathy (p = 0.0004, Fischer's exact test).

MOTOR	No AN	AN +
No Motor Nerve	14	18
Any – inelicitable /abnormal	21	71

p = 0.02 (Chi square)

MOTOR	No / Mild/ Moderate AN	Severe AN +
No Motor Nerve or < 4 inelicitable /abnormal	69	0
4 or more inelicitable /abnormal	46	9

p = 0.0004 (Fischer's exact test)

The correlation with sensory nerve abnormalities was weaker though still statistically significant. Presence of 3 or more abnormal or inelicitable sensory nerves increases the risk for severe autonomic neuropathy (p = 0.048).

DISCUSSION

Diabetic neuropathies are the most common types of neuropathies worldwide. Duration and degree of metabolic control are the 2 major predictors in the development of neuropathy and determinant of its severity. Our study group mainly consisted of patients with type 2 diabetes except for two patients who had maturity onset diabetes of young. 115 patients with various degrees of diabetic peripheral neuropathy (classified into mild, moderate and severe) and 9 with CTS (who did not have any diabetic peripheral neuropathy) were taken their autonomic dysfunction was systematically analysed. The mean duration of diabetes was 12.02 years for the entire group. Bivariate analysis had shown a markedly significant correlation between the duration with severity of peripheral neuropathy with mild having a disease duration of 9.17 yrs, moderate 11.60 yrs and severe with 17.98 years. The same markedly significant correlation held true for the duration of symptoms and severity of peripheral neuropathy. Age did not have any correlation with either peripheral or autonomic neuropathy. Dyck et al. has reported that longer duration of diabetes also increases the possibility of developing more than one form of diabetic neuropathy(55).This factor is exemplified by a threefold increase in the prevalence of sympathetic and parasympathetic neuropathies in patients with diabetic neuropathy 10 years after the initial diagnosis of neuropathy(55).In our study the duration of any neuropathic symptoms had a significant correlation with severity of autonomic neuropathy while duration of diabetes did not. Patients with mild autonomic neuropathy had disease duration of 10.84 yrs, moderate 12.80 yrs and severe had 16.67 years but this did not attain statistical significance because the subgroup with no autonomic neuropathy had disease duration of 11.18 years.

Genetic factors play a role in individual susceptibility to diabetic neuropathy. APO-E genotype has been proposed as a risk factor for the severity of neuropathy in patients who have diabetes (56). Having an E3/E4 and 4/4 APOE genotype is the equivalent of having 15 extra years of age or diabetes duration. However the effect of this genotype is marked in type I DM and less so in type

2(57). It can influence the severity of neuropathy by several mechanisms including acceleration of atherosclerosis, changes in cell adhesion or use of growth factors (58). There are earlier studies where they have found that autonomic dysfunction manifesting as early as after detection of diabetes itself 20).

The Rochester Diabetic Neuropathy Study (59), which prospectively studied 380 of 870 patients who had diabetes in Rochester, Minnesota, revealed that 278 patients (73.2%) had type II diabetes, whereas 102 patients (26.8%) had type I diabetes. Fifty-nine percent of patients who had type II and 66% of patients who had type I had some forms of neuropathy. Rochester study was on a community group diagnosed with diabetes(59). They had detected the prevalence of autonomic neuropathy to be 7% in Type I and 5% in Type II. We had 36 patients with mild 50 with moderate and 29 with severe peripheral neuropathy. Out of them 35 did not have any evidence for autonomic involvement 40 had mild, 40 moderate and 9 had severe autonomic neuropathy.

40 patients (35%) had autonomic symptoms the most common being impotence and postural dizziness (presyncope /syncope). McCulloch et al has reported erectile dysfunction (ED) to be the most common and earliest form of organic dysfunction in males with diabetes, with an incidence estimated to be between 35 and 75% (60). Ejaculatory dysfunction may occasionally preceed erectile dysfunction but is less often reported (61). Valsalva ratio was abnormal in 76 patients (61.3%) followed by E/I ratio abnormal in 60 patients (48.4%). 30/15 ratio was the least sensitive of autonomic cardiac tests. SSR was abnormal only in 12 patients (12.9%) suggesting that electrophysiological detection of sudomotor dysfunction occurs later in the course of illness.

Albuminuria is a strong risk factor for neuropathy in type 2 DM (62). In our study 78% of the patients with mild peripheral neuropathy had no albuminuria while 14% with mild disease had only trace albuminuria. 25% of those with severe peripheral neuropathy had either 2+ or 3+ albuminuria. These values were highly significant .1/3 of patients with no autonomic neuropathy did not have

albuminuria while 80% of those with 3+ albuminuria had either moderate or severe autonomic neuropathy.

The correlation of autonomic neuropathy and other end organ damage was equally strong. 74% of patients with no retinopathy had either no autonomic neuropathy or only a mild disease where as 53% of those with proliferate retinopathy had moderate to severe autonomic neuropathy.

Analysis of the presentation pattern by NSS showed that sensory symptoms were the most common seen in 107 of 115 with peripheral neuropathy. This is particularly relevant and one should always ask "Is it diabetic neuropathy or neuropathy in a diabetic patient?" when the neuropathy is rapidly progressive, there is prominent motor abnormality or cranial nerve involvement, or there are disproportionate large fiber abnormalities. If there is involvement of the entire lower limbs without neuropathy of the distal upper limbs, it is unlikely that the process would be related to diabetic neuropathy and other causes should be investigated. It has been estimated that approximately one third of patients who have diabetes have a neuropathy unrelated to diabetes (63). In The Rochester Diabetic Neuropathy Study (59), 10% of patients who have diabetes and distal sensory neuropathy had other possible causes of neuropathy.

One of the hallmarks features of the current study was an attempt to predict autonomic neuropathy based on peripheral neuropathy findings. Previous studies have had differing opinions. The main fibre types affected in diabetic neuropathy are the small fibres C and Aδ early in the disease course which are not picked up by routine NCS which test the large diameter myelinated fibres. Therefore autonomic dysfunction may be picked up earlier by standardized autonomic function tests before peripheral neuropathy is picked up in routine nerve conduction studies. In our study logistic regression analysis of the severity of autonomic neuropathy Vs various nerve conduction study parameters revealed that the number of abnormal motor nerves had the best correlation. Presence of even one abnormal motor nerve increases the risk of mild autonomic neuropathy ($P = 0.02$) while the presence of 4 or more abnormal/ inelicitable motor nerves increases the risk for severe autonomic neuropathy ($P = 0.0004$). The correlation

with sensory nerve abnormalities was weaker though still statistically significant. Presence of 3 or more abnormal or inelicitable sensory nerves increases the risk for severe autonomic neuropathy (P = 0.048). So our study has given a model for the prediction of autonomic neuropathy when only routine nerve conduction parameters are available.

Implications Of Cardiovascular Autonomic Neuropathy: In our study severity of autonomic neuropathy strongly correlated with coronary artery disease ($p<0.001$) 62.5% of CAD patients on drugs and 100% of those who had undergo CABG had moderate or severe autonomic neuropathy.48% of patients with severe peripheral neuropathy had coronary artery disease (CAD) and were on drugs or stent or has undergone CABG while only 30.5% of patients with mild neuropathy had CAD but these were not statistically significant ($p=0.055$). Identifying individuals at risk is only the first step in managing patients and ultimately affecting outcomes. After identification, proactive measures are required, because if those patients at high risk or those shown to be in early stages are not treated until advanced symptomatology is present, little has been achieved.

Tests that provide evidence of further health consequences may bring patients to medical attention before other signs of diabetic end-organ injury emerge, making proactive treatment, particularly the establishment of intensive diabetes care, possible. The results of autonomic function testing can contribute to good patient management.

To assist in the establishment (or reestablishment) of tight glycemic control: Early observations by researchers that near-normal glycemic control seems to be the most effective way to delay the onset of CAN in type 1 diabetes has been confirmed by evidence from the DCCT (49). Intensive insulin therapy has been shown to be effective at preventing multiple complications in patients with type 1 diabetes and is postulated to be effective for patients with type 2 diabetes. In our study HbA1c levels and severity of diabetic peripheral neuropathy had a strong correlation ($P<0.001$). Those with mild peripheral neuropathy had a mean HbA1c of 5.986 gm%, moderate had 6.876 gm% and

severe had 7.769 gm%. The severity of peripheral neuropathy and FBS/PPBS values had a significant corelation p=0.001. The mild peripheral neuropathy group had a FBS/PPBS of 107.6 / 158.4 mgm%, moderate had 131.62 /169.3 mgm% and severe had 144.55/211.17mg%.

HbA1c had a strong correlation with the severity of diabetic autonomic neuropathy (P<0.001). Those with no autonomic neuropathy had a HbA1c of 6.314 gm% while those with severe had 7.833 gm%. FBS values correlated with severity of autonomic neuropathy but were statistically not significant (p=0.085). But the correlation between PPBS and total cholesterol with autonomic neuropathy were statistically significant (p=0.018 and 0.036 respectively). PPBS of those with no autonomic dysfunction were 148.83 mg% while those with severe autonomic involvement being 203.56 mg%. Logistic regression analysis of presence autonomic neuropathy was assessed and among them autonomic neuropathy had a strong correlation with PPBS, coronary artery disease, and neuropathy disability score (NDS).

In its earliest stages, there has been some clinical demonstration that autonomic dysfunction may be influenced within a few days to a few weeks with effective treatment (64). Delay in instituting appropriate interventions can only increase the likelihood of developing advanced neuropathies. Stabilization of the neuropathies (generally considered to be any delays in further progression) through tight glycemic control seems possible, whereas reversal of the condition may be less likely (65). Again, the results from the DCCT show that intensive glycemic treatment can prevent the development of abnormal heart rate variation and slow the deterioration of autonomic dysfunction over time for individuals with type 1 diabetes (49). Tight control for individuals with autonomic dysfunction should also include increased vigilance in glycemic monitoring and reeducation of the patient with regard to hypoglycemia.

To facilitate the decision to initiate treatment for cardiovascular autonomic dysfunction: Study revealed that severity of autonomic neuropathy correlated strongly with coronary artery disease. Logistic regression analysis of presence

autonomic neuropathy had a strong correlation with PPBS, coronary artery disease, and neuropathy disability score (NDS). Severe autonomic neuropathy was strongly associated with coronary artery disease, severity of peripheral neuropathy and urine albuminuria.

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 nonpharmacological 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. Interventions to modulate reduced heart rate variation currently being studied in clinical trials are based on theories of the pathogenesis of CAN(66).

Evidence from clinical trials evaluating the use of antioxidants is promising. Early identification of CAN permits timely initiation of therapy with the antioxidant alpha-lipoic acid (thioctic acid), which appears to slow or reverse progression of neuropathies in some studies (67), but further testing is necessary. Studies using ACE inhibitors as a means to improve heart rate variation have resulted in conflicting results. Quinapril significantly increased parasympathetic activity after 3 months of treatment (68), whereas cardiovascular autonomic function did not change significantly after 12 months of treatment with trandolapril (69).

The use of cardioselective (e.g., atenolol) or lipophilic (e.g., propranolol) ß-blockers may also modulate the effects of autonomic dysfunction (1). By opposing the sympathetic stimulus, they may restore the parasympathetic-sympathetic balance. Recently, the administration of metoprolol to ramipril-treated type 1 diabetic patients with abnormal albuminuria has been shown to improve autonomic dysfunction (70).

The pathogenesis of CAN is most likely a multifactorial process, a combination of therapies directed simultaneously at different parts of the pathogenic pathway may be needed. In addition, the goal of these interventions

should be directed at the prevention of further deterioration of cardiovascular autonomic dysfunction rather than expecting to realize improved function.

To emphasize the importance of adherence to diet and exercise interventions: In this study the severity of diabetic autonomic neuropathy did not correlate with BMI. Though the 35 patients with no autonomic neuropathy had a BMI of 25.03 and the moderate autonomic neuropathy group (40 patients) had a BMI of 25.40 these were not statistically significant (p=0.444). But there are studies stating that lifestyle interventions (e.g., adherence to diet and exercise) can reduce the incidence of type 2 diabetes (71). Recently, a report indicated that impaired glucose tolerance may be associated with the development of diabetic neuropathy (i.e., sensory polyneuropathy) (72).The ability to determine early stages of autonomic dysfunction could intensify the salience of measures such as diet and exercise that directly affect efforts to establish tight glycemic control and delay the development of autonomic dysfunction. Howorka et al. (73) showed that physical training improved heart rate variation in insulin-requiring diabetic individuals with early CAN.

Silent myocardial ischemia/cardiac denervation syndrome: A reduced appreciation for ischemic pain can impair timely recognition of myocardial ischemia or infarction and thereby delay appropriate therapy. Of the 12 studies, 5 showed a statistically significant increased frequency of silent myocardial ischemia in individuals with CAN compared with individuals without CAN. These data demonstrate a consistent association between CAN and the presence of silent myocardial ischemia. The presence of CAN does not exclude painful myocardial infarction (MI) among individuals with diabetes. (74).

Increased risk of mortality: Studies have consistently provided evidence for an increased mortality risk among diabetic individuals with CAN compared with individuals without CAN. Ewing et al. (27) reported a 2.5-year mortality rate of 27.5% that increased to 53% after 5 years in diabetic patients with abnormal autonomic function tests compared with a mortality rate of only 15% over the 5-

year period among diabetic patients with normal autonomic function test results. It should be noted that half of the deaths in individuals with abnormal autonomic function tests were from renal failure, and 29% were from sudden death. This study also revealed that symptoms of autonomic neuropathy, especially postural hypotension, and gastric symptoms in the presence of abnormal autonomic function tests carried a particularly poor prognosis. O'Brien et al., compared the relative importance of various factors associated with mortality by discriminate analysis of survivors and nonsurvivors using Rao's stepwise selection method and revealed that autonomic neuropathy was more of an independent predictive factor than systolic blood pressure, foot disease, BMI, sensory neuropathy, proteinuria, and macrovascular disease (75)

Rathmann et al. (76) reported the results of a study designed to assess the risk of mortality due to CAN among patients with CAN but without a clinical manifestation of severe complications (proteinuria, proliferative retinopathy, coronary artery disease, or stroke) 8 years after their first clinical examination. The mortality of diabetic patients with CAN increased steadily over the 8-year period (6% after 2 years, 14% after 4 years, 17% after 6 years, and 23% after 8 years) compared with an age-, sex-, and duration of diabetes-matched control group where there was one death.

Meta-analysis of the relationship between CAN and mortality: In all there were 15 studies, where the baseline assessment for cardiovascular autonomic function was made on the basis of one or more of the tests described by Ewing et al(27). Total mortality rates were higher in subjects with CAN at baseline than in subjects whose baseline assessment was normal, with statistically significant differences in 11 of the studies.

Association of CAN with major cardiovascular events: Earlier studies have examined 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 the need for

coronary revascularization. The relative risks associated with CAN in these studies were 2.2 and 3.4, respectively, with the latter result just achieving statistical significance ($P < 0.05$).

Potential reasons for the increased mortality rate associated with CAN

Several mechanisms have been suggested including a relationship with autonomic control of respiratory function. Page and Watkins (77) reported 12 cardiorespiratory arrests in eight diabetic individuals with severe autonomic neuropathy and suggested that diabetic individuals with CAN have impaired respiratory responses to conditions of hypoxia and may be particularly susceptible to medications that depress the respiration system. An impaired ability to recognize hypoglycemia and impaired recovery from hypoglycemic episodes due to defective endocrine counterregulatory mechanisms are also potential reasons for death. Other investigators have noted explanations for the high mortality rate as an interaction with other concomitant disorders that also carry high risks of mortality. Clarke et al. (6) speculated that the increased mortality found for patients with clinical symptoms of autonomic neuropathy were due to both a direct effect of the autonomic neuropathy itself and an indirect, but parallel, association with accelerating microvascular complications. O'Brien et al. (75) suggested that the high rate of mortality due to end-stage renal disease among diabetic patients with autonomic neuropathy may have been due to the parallel development of late-stage neuropathy and nephropathy. The presence of autonomic neuropathy may accelerate the rate of progression of diabetic glomerulopathy by mechanisms not completely understood. A consequential increase in cardiovascular risk experienced by individuals with nephropathy has also been noted. In one study of type 1 diabetic individuals, hypertension along with LDL and HDL cholesterol concentrations were found to be independent correlates of CAN. These results suggested that a disturbed cardiovascular risk profile seen in individuals with nephropathy might lead to both cardiovascular disease and CAN. Other investigators have also shown independent associations of autonomic dysfunction with markers of cardiovascular risk (e.g., elevated blood

pressure' body weight, glycosylated hemoglobin, and overt albuminuria). Nonetheless, CAN cosegregates with indexes of macrovascular risk, which may contribute to the marked increase in cardiovascular mortality. Diabetic patients with CAN are predisposed to a lack of the normal nighttime decrease in blood pressure because of an increased prevalence of sympathetic activity. A disturbed circadian pattern of sympathovagal activity with prevalent nocturnal sympathetic activity combined with higher blood pressure values during the night and increased left ventricular hypertrophy could represent another important link between CAN and an increased risk of mortality.

CAN and sudden death: One potential cause of sudden death among subjects identified with autonomic neuropathy may be explained by severe but asymptomatic ischemia, eventually inducing lethal arrhythmias· An autonomic imbalance resulting in QT prolongation may also predispose individuals to life-threatening cardiac arrhythmias and sudden death. Katz et al. (78) showed that a simple bedside test that measured 1-min HRV during deep breathing was a good predictor of all-cause mortality for 185 patients (17.8% with diabetes) after a first MI. These investigators also suggested that cardiovascular autonomic function testing provided a predictive value that could be used to identify a subgroup of patients after an MI who are at a high risk for cardiovascular death.

Association of cerebrovascular disease and CAN: Severity of diabetic peripheral neuropathy assessed against cerebrovascular disease as evidenced by history of TIA or carotid stenosis by doppler did not reveal any statistical significance (p= 0.378). This was because of the fact that most stroke patients had to be excluded from the study due to their inability to perform autonomic tests.

The impact of autonomic dysfunction on the risk of the development of strokes was examined by Toyry et al. (79), who followed a group of 133 type 2 diabetic patients for 10 years. During the study period, 19 individuals had one or

more strokes. Abnormalities of parasympathetic and sympathetic autonomic function were found to be independent predictors of stroke in this cohort .

Hypoglycemic unawareness and DAN: DAN plausibly could cause or contribute to hypoglycemia unawareness. Ryder et al. observed that patients with autonomic neuropathy had a negligible plasma pancreatic polypeptide response (3.7 pmol/l), and this response was also blunted in the patients with inadequate hypoglycemic counterregulation (72.4 pmol/l) compared with that of the control subjects (414 pmol/l; $P < 0.05$) (80). More recent data suggest that the presence of autonomic neuropathy further attenuates the epinephrine response to hypoglycemia in diabetic individuals after recent hypoglycemic exposure (81).

Relationship of autonomic neuropathy to tissue perfusion: Microvascular skin flow is under the control of the ANS and is regulated by both the central and peripheral components (82). In diabetes, the rhythmic contraction of arterioles and small arteries is disordered. Microvascular insufficiency may be a cause of diabetic neuropathy (83). Microvascular blood flow can be accurately measured noninvasively using laser Doppler flowmetry (84).

Progression of CAN

Results of the cardiovascular autonomic function tests that are mediated mainly by the parasympathetic nervous system (e.g., heart rate response to deep breathing) are typically abnormal before those responses that are mediated by the sympathetic nerves. Although one might speculate then that parasympathetic damage occurs before sympathetic damage, this may not always be true. In our study valsalva was shown to be more sensitive than other tests for early detection of autonomic dysfunction. The increased frequency of abnormalities detected via tests of the parasympathetic system may merely be a reflection of the test (e.g., sensitivity) and not of the natural history of nerve fiber damage. Thus, it may be better to describe the natural history of autonomic

dysfunction as developing from early to more severe involvement rather than to anticipate a sequence of parasympathetic to sympathetic damage (85).

WHO IS A CANDIDATE FOR TESTING?

Evidence from clinical literature can be found that support testing recommendations for various subpopulations. They include the following.

Diabetic patients with a history of poor glycemic control: Long-term poor glycemic control can only increase the risk of developing advanced diabetic neuropathy, although long-term follow-up studies are lacking. Mustonen et al. (88) showed in a 4-year follow-up study of 32 individuals with type 2 diabetes that poor glycemic control was an important determinant of the progression of autonomic nerve dysfunction. The DCCT provided extensive clinical evidence that good metabolic control reduces diabetic complications. Specifically with regard to cardiovascular autonomic function, the DCCT showed that intensive glycemic control prevented the development of abnormal heart rate variation and slowed the deterioration of autonomic dysfunction over time for individuals with type 1 diabetes (49). Unfortunately, however, one cannot predict what the metabolic control will be (or has been) over a long period of time by looking at current HbA_{1c} results.

Poor glycemic control may also be a consequence of DAN (e.g., gastroparesis that goes unidentified). Treatment of GI dysfunction often improves glycemic control.

Diagnosed diabetic patients

Researchers have confirmed the presence of autonomic neuropathy at presentation (20). Some manifestations of autonomic neuropathy may even precede the diagnosis of diabetes by several years (89). Testing for cardiovascular autonomic dysfunction is suggested for individuals with diabetes. This includes testing to identify children and adolescents with autonomic neuropathy.

Although much remains to be learned about the natural history of CAN, previous reports can be coalesced into a few observations that provide some insight with regard to progression of autonomic dysfunction:

- It can be detected at the time of diagnosis (43).
- Neither age nor type of diabetes are limiting factors in its emergence, being found in young individuals with newly diagnosed type 1 diabetes and older individuals newly diagnosed with type 2 diabetes (43).
- Poor glycemic control plays a central role in severity and progression (86,current study).
- Intensive therapy can slow the progression and delay the appearance of abnormal autonomic function tests (49).
- Subclinical autonomic neuropathy can be detected early using autonomic function tests (23, current study).
- Autonomic features that are associated with sympathetic nervous system dysfunction (e.g., orthostatic hypotension) are relatively late complications of diabetes (27, current study).
- There is an association between CAN and diabetic nephropathy which may contribute to high mortality rates (27, current study).

Even with consensus regarding these general observations, much remains unclear:

- Type 1 and type 2 diabetes may have different progression paths.
- The relationship between autonomic damage and duration of diabetes is not clear although numerous studies support an association (86, 87).
- Prevalence and mortality rates may be higher among individuals with type 2 diabetes, potentially due in part to longer duration of glycemic abnormalities before diagnosis.

SUMMARY

Autonomic dysfunction is a prevalent and serious complication for individuals with diabetes. The clinical manifestations of autonomic dysfunction can affect daily activities (e.g., exercise), produce troubling symptoms (e.g., syncope), and cause lethal outcomes. The patient's history and physical examination are ineffective for early indications of autonomic nerve dysfunction, and thus recommendations for the use of noninvasive tests that have demonstrated efficacy are warranted. The relative cost of testing will always be less than the incremental costs of treating either a detected complication or the more catastrophic event that could eventually occur.

Current study was done on a total of 124 diabetic individuals who did not have any other alternate cause for neuropathy. The autonomic function tests were done as described by Ewing et al .The mean age of the study population was 59.62 yrs with a minimum of 34 and maximum of 82 years of whom 71% were males. The mean body mass index was 25.024 while the mean duration of diabetes was 12.02 years. Females were more likely to be either over weight or obese than males. Only 1/3rd of the patient group (33.9%) had good glycemic control. Mean levels of glycosylated haemoglobin level were 6.737 gm % and 40.3% patients had good control. 42% of the patients had evidence for early nephropathy in the form of presence of urine albuminuria while 28.2% had a history of coronary artery disease and 50.8% had evidence of diabetic retinopathy.

Among the tests for heart rate variability valsalva was the most sensitive being abnormal in 61.3% closely followed by expiration: inspiration ratio which was abnormal in 48.4%. 72.8% patients had evidence for autonomic neuropathy of which 7.3% patients had severe autonomic neuropathy while 32.3% had mild and a similar percentage had moderate involvement.

Patients with mild peripheral neuropathy had mean diabetes duration of 9.17 years, those with moderate 11.60 years and those with severe 17.98 years. Duration of neuropathic symptoms and severity of peripheral neuropathy had a strong correlation. Those with mild peripheral neuropathy had a mean HbA1c of 5.986 gm%, moderate had 6.876 gm% and severe had 7.769 gm%.

The severity of diabetic peripheral neuropathy and autonomic neuropathy had a strong correlation. 76% of those with severe peripheral neuropathy had either moderate or severe autonomic neuropathy while among those with mild peripheral neuropathy 33.3% had no autonomic involvement and 44.4% had only mild autonomic neuropathy. 74% of patients with no retinopathy had either no autonomic neuropathy or only a mild disease where as 53% of those with proliferate retinopathy had moderate to severe autonomic neuropathy.1/3 of patients with no autonomic neuropathy did not have albuminuria while 80% of those with 3+ albuminuria had either moderate or severe autonomic neuropathy. Severity of autonomic neuropathy strongly correlated with coronary artery disease. 62.5% of CAD patients on drugs and 100% of those who had undergone CABG had moderate or severe autonomic neuropathy.

Multivariate analysis showed that severe autonomic neuropathy was strongly associated with coronary artery disease, severity of peripheral neuropathy and urine albuminuria. Severity of peripheral neuropathy showed corelation to duration of symptoms, HbA1c, and total neuropathic disability score.

One of the hallmarks features of the current study was an attempt to predict autonomic neuropathy based on peripheral neuropathy findings. In our study revealed that the severity of autonomic neuropathy had the best correlation Vs the number of abnormal motor nerves by routine nerve conduction studies. Presence of even one abnormal motor nerve increases the risk of mild autonomic neuropathy while the presence of 4 or more abnormal/ inelicitable motor nerves increases the risk for severe autonomic neuropathy.

The correlation with sensory nerve abnormalities was weaker though still statistically significant. Presence of 3 or more abnormal or inelicitable sensory nerves increases the risk for severe autonomic neuropathy. So our study has given a model for the prediction of autonomic neuropathy when only routine nerve conduction parameters are available.

Given the clinical and economic impact of diabetic autonomic neuropathy, testing of diabetic individuals for cardiovascular autonomic dysfunction should be part of their standard of care.

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