COMPARISON OF STANDARD OUTPATIENT SCREENING TOOLS AND NERVE CONDUCTION STUDIES FOR THE DIAGNOSIS OF DIABETIC PERIPHERAL NEUROPATHY – A PILOT STUDY

Dissertation submitted to the Tamil Nadu Dr M.G.R Medical University, Chennai, Tamil Nadu, in partial fulfilment of the requirements for the MD branch XIX (Physical Medicine and Rehabilitation) University Examinations in April 2017

CERTIFICATE

I hereby certify that the dissertation titled **" COMPARISON OF STANDARD OUTPATIENT SCREENING TOOLS AND NERVE CONDUCTION STUDIES FOR THE DIAGNOSIS OF DIABETES PERIPHERAL NEUROPATHY"** is my bona fide work in partial fulfillment of the requirement of the Tamil Nadu Dr. MGR University, Chennai, for the MD branch XIX (Physical Medicine and Rehabilitation) for university examinations in April 2017.

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This is to certify that the thesis tilted **"Comparison of standard outpatient screening tools and nerve conduction studies for the diagnosis of diabetic peripheral neuropathy – a pilot study"** is the bonefide work of **Dr Saraswathi Ramanathan**, candidate number **201429053** in fulfilment of the requirement of the Tamil Nadu Dr M.G.R Medical University, Chennai, Tamil Nadu for the MD branch XIX (Physical Medicine and Rehabilitation) University Examinations in April, 2017.

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Comparison of standard outpatient screening tools and nerve conduction studies for the diagnosis of diabetic peripheral neuropathy - a pilot study
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Match Overview
REVIEW OF LITERATURE:
CLINICAL FEATURES AND COMPLICATIONS OF TYPE 2 DIABETES MELLITUS

ACKNOWLEDGEMENT:

At the outset, I would like to offer my humble thanks to the Lord Almighty, who has been with me and guided me throughout this journey. I would like to thank all the healthy volunteers and my patients who consented and participated in the study. Nothing can be complete without the meticulous guidance of beloved teachers. I would like to express my heartfelt gratitude to my guide Dr. Raji Thomas, (Professor and Head, Department of PMR, CMC, Vellore) for having spent her valuable time with me whenever I needed her, helping me to gain a meaningful approach to the study and moulding the project to the present shape. I am thankful to Dr. Nihal Thomas, Professor and Head, Department of Endocrinology, CMC, Vellore, and my Co-Guide for offering his valuable time, knowledge and experience. I thank Dr.Asem Rangita Chanu(Assistant Professor, Department of PMR and my co-investigator) and Dr. Navin (Assistant Professor, Department of PMR) for their guidance while performing nerve conduction studies. Iwould like to thank Dr. Prashanth Chalegeri(Assistant Professor, Department of PMR, and my co-investigator) and Dr. Dukhbandu Naik(Assistant Professor, Department of Endocrinology) for their support and guidance. I am grateful to all consultants, co-registrars and friends for their constant support and help throughout the study. Not to forget,special thanks to my statistician Mrs. Gowri for her inputs on statistical analysis. Last, but not the least, I would like to thank my parents and family, for their constant prayers and good wishes.

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INTRODUCTION:

Diabetes Mellitus is a metabolic disorder primarily affecting the neurovascular system. Currently, it is a worldwide problem, becoming an impending epidemic in India. According to the WHO, the term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both.(1)

The effects of diabetes mellitus include long-term micro and macro vascular complications. Diabetes and its complications together constitute an extensive burden on the health care system, especially in a developing country like India where resources for management are few. Therefore, optimal control of diabetes along with early diagnosis and management of complications is very important.

Diabetic peripheral neuropathy, which is the most common complication, if not diagnosed and managed well, can lead to foot ulcers, Charcot joint and ultimately loss of limb. All these contribute towards significant mortality, morbidity and economic burden. Early diagnosis gives the opportunity for the patient to optimize glycemic control and implement better foot care before the onset of significant morbidity. Hence it becomes essential to assess for these complications at routine intervals with appropriate screening tools.

A number of measuring tools are used for assessment of neuropathy ranging from different questionnaires, monofilament testing, biothesiometer and nerve conduction studies. Till date there is no consensus on the gold standard tool for assessment of neuropathy. In this study we try to compare the various screening tools used in diabetic peripheral neuropathy.

AIM& OBJECTIVES

AIM:

To study the usefulness of clinical testing as compared to Nerve conduction studies for the early detection of sensorimotor polyneuropathy in patients with Type 2 Diabetes Mellitus.

OBJECTIVES:

1. To study the occurrence of peripheral neuropathy in patients with diabetes mellitus by using standard outpatient clinical tools and nerve conduction studies

2. To compare the results of nerve conduction studies, Semmes Weinstein monofilament testing and vibration perception testing using biothesiometer with the results of MNSI (Michigan Neuropathy Screening Instrument) in the detection of diabetic sensorimotor polyneuropathy.

3. To compare the results of biothesiometer testing with Nerve conduction studies in detection of diabetic sensorimotor polyneuropathy.

4. To assess the usefulness of minimal F wave latency and sural radial amplitude ratio (SRAR) in early detection of diabetic polyneuropathy

5. To study the sensitivities and specificities of the various screening instruments used to detect diabetic peripheral neuropathy based on nerve conduction studies as the gold standard

REVIEW OF LITERATURE:

Diabetes Mellitus is on the verge of becoming an epidemic in India, and currently has the highest number of cases. With increasing urbanization, prevalence of obesity and physical inactivity have gone up and there is an increase in the number of people with diabetes. According to the 2014 data, there are 387 million people with diabetes in the world and it has been estimated that 592 million people will be affected by 2035.(2) According to the International Federation of Diabetes, India has the highest number of Diabetics in the world. According to the current statistics in India, the prevalence of Diabetes is 62 million, and 100 million people are estimated to suffer from Diabetes mellitus by the year 2030. (3)

The true prevalence of diabetic neuropathy is not known. Various studies suggest the prevalence to vary from 10-90%.(4) This could be attributed to the fact that different screening tools are used for detecting diabetic neuropathy. The other significant factors contributing to this variation are the age of the patient and the time lapsed before diagnosis. A prospective study to detect the prevalence of diabetic neuropathy in newly diagnosed diabetics, in Northern India showed a prevalence of around 30%.(5,6) Another study done on 1629 diabetics in South India showed a similar prevalence of 26.1%.(7)This study aims to compare the results of various screening tests to detect peripheral neuropathy in patients with diabetes mellitus.

CLINICAL FEATURES AND COMPLICATIONS OF TYPE 2 DIABETES MELLITUS:

Patients with Diabetes Mellitus may initially present with polydipsia, polyuria, blurring of vision, and weight loss. However, many a time, symptoms are not severe and the silent hyperglycemia may cause pathological functional changes before the diagnosis is made. Acute and Chronic complications may occur. The acute complications include ketoacidosis or a non-ketotic hyperosmolar state. The chronic complications include retinopathy, nephropathy, and neuropathy with risk of foot ulcers, amputation, Charcot joints, features of autonomic dysfunction, increased risk of cardiovascular, peripheral vascular and cerebrovascular disease.(1)14.3% of the diabetics have foot ulcers.(8) The incidence is higher in India than in the Western population and this could be attributed to various social and cultural practices of barefoot walking. Low socio-economic status and illiteracy leads to inappropriate usage of footwear and increasing incidence of foot ulceration(9). Foot ulcers precede over 85% of lower limb amputations. Diabetes is the major cause of non-traumatic amputation across the world, rates of which are 15 times higher as compared to amputation rate among non-diabetic population.(10) Another study showed that around 10.5% of diabetics underwent major amputations and the postoperative mortality was 14.7%. (11)

DIABETIC PERIPHERAL NEUROPATHY- DEFINITION AND TYPES:

Diabetes mellitus is associated with various neuropathy syndromes that differ in their aetiology, natural history, and treatment. Diabetic neuropathy can be defined as the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes.(12)

As with other complications of diabetes mellitus, the development of neuropathy correlates with age, anthropometric measures like height of the patient, duration of diabetes and glycemic control. Neuropathy can be broadly divided into symmetric and asymmetric types, although a great deal of overlap exists between these categories. The pattern and the symptomatology depend on the type of nerve fibres involved. Figure 1 shows the different types of nerve fibres of the peripheral nervous system and outlines the symptoms attributed to each type of fibre.

Symmetric neuropathies may present as small-fibre or large fibre involvement or autonomic dysfunction. Asymmetric Diabetic Neuropathy includes cranial neuropathies, limb mononeuropathies, radiculopathies, plexopathies, and diabetic amyotrophy.

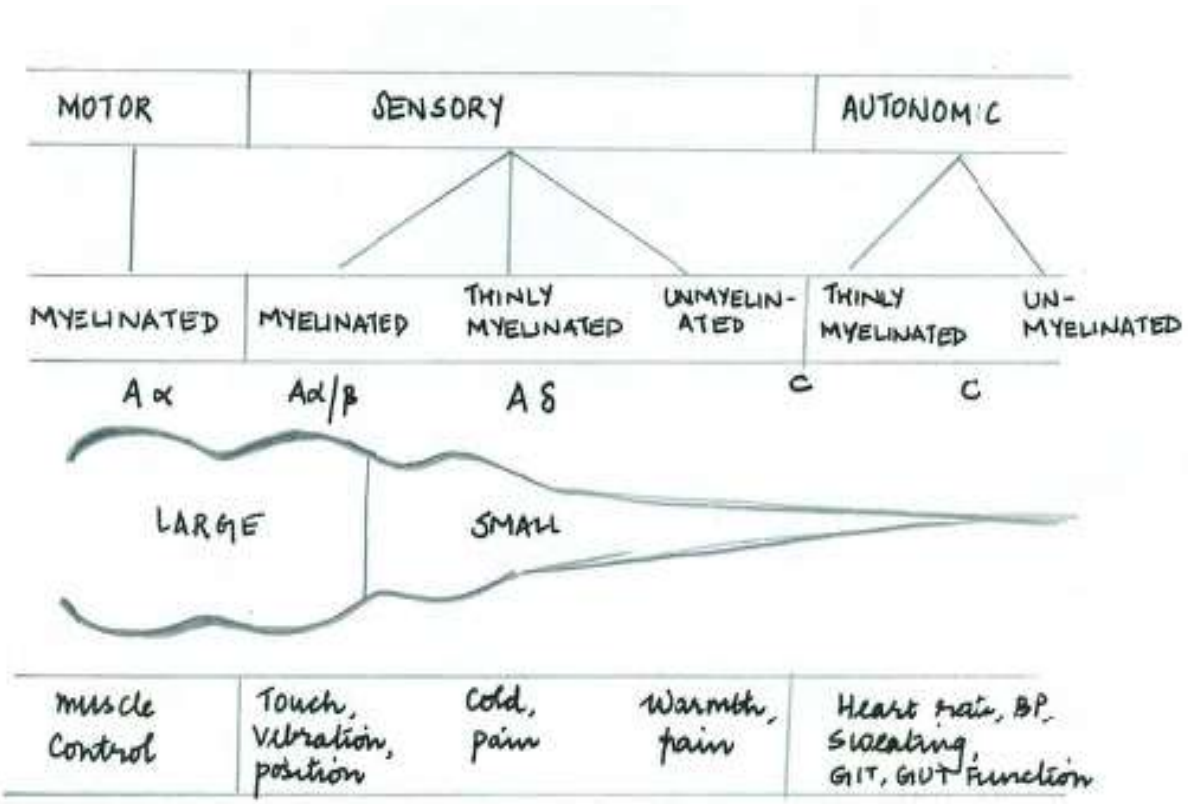


Figure 1 - Simplified view of the peripheral nervous system

The types of Diabetic neuropathy are outlined below:

Rapidly reversible hyperglycaemic neuropathy:

This is characterised by reversible distal sensory symptoms in patients with recently diagnosed diabetes or in those with a poor glycemic control. When they reach a euglycemic state, recovery tends to occur.

Diabetic Sensorimotor Polyneuropathy:

Length-dependent sensorimotor polyneuropathy (DSPN) is the most common type of diabetic neuropathy accounting to around 80%(13). It can be found at the time of diagnosis of Type 2 diabetes itself. This type is predominantly distal and symmetric. It is a mixed neuropathy with small- and large-fibre, sensory, autonomic, and motor nerve involvement in various combinations, with sensory and autonomic symptoms more prominent than motor ones. Length dependent diabetic polyneuropathy usually starts at the feet and progresses proximally, and as it reaches the knee, symptoms would start in the distal aspects of the hand, progressing proximally in both upper and lower limbs. When it reaches the most proximal part of the lower limbs, it can progress to the anterior aspect of the trunk, involving the sensory component of the intercostal nerves. This indicates that the neuropathy is length dependent.

Symptoms can range from being completely clinically silent to symptoms like pain, hyperesthesia, parasthesia, burning and tingling. They can also present with negative symptoms like and numbness, painless foot ulcers, subsequently leading to amputation. They may occasionally present with unsteadiness due to abnormal proprioception and decreased sensation. The clinically silent variety can be detected only by examination.

In Diabetes, there is a loss of both myelinated and unmyelinated nerve fibers. There can be an involvement of both small and large fibres. Small fibre neuropathy presents with reduced intra-epidermal nerve fibre density. The first modalities to be affected are pain and temperature. Loss of large myelinated fibrescan cause disturbance of light touch, vibration and joint position sense. (Figure 1) Motor involvement occurs at a later stage, only when there is profuse sensory involvement, and is not quite common.

Neuropathic pain can occur and is a disabling condition if present. It is more common in small fibre neuropathy with intra-epidermal nerve fibre loss. Trophic changes can also occur in symmetric sensory polyneuropathy. They first present with callus formation or a painless phlyctenular lesion(14). This is followed by bullous lesions and plantar ulcers. The ulcers can progress and lead to an ankle joint arthropathy. However, ulceration and arthropathy are not only limited to diabetes. Any condition causing loss of sensation of the feet, like leprosy, meningomyelocoele, hereditary sensory and alcoholic sensory neuropathies can also result in arthropathy.

Diabetic Autonomic Neuropathy:

Autonomic dysfunction is one of the serious manifestations of neuropathy, which often co-exist with small fibre neuropathy. It has a significant negative impact on

survival.(12) It has a varied presentation including the gastrointestinal, cardiovascular and genito-urinary symptoms.

Focal and Multifoal Neuropathies:

Focal and multifocal neuropathies are much less common. They include entrapment neuropathies, mononeuropathies, cranial nerve neuropathies, proximal diabetic neuropathy of lower limbs and limb and truncal neuropathies.

Mononeuropathies are usually of acute onset, involve the median nerve(5.8% of diabetic neuropathies), ulnar nerve(2.1%), radial nerve(0.6%) and common peroneal nerves(15). They are associated with pain, however have a self limiting course.

Entrapment syndromes differ from mononeuropathies in that they have a gradual onset, are progressive and persist if intervention is not done. Carpel tunnel syndrome is a common entrapment neuropathy. A study done by Perkins et al showed that the prevalence of CTS was 2% in the reference population (without diabetes and without neuropathy), 14% in diabetics without polyneuropathy and 30% in those with diabetic polyneuropathy. (16)

Cranial neuropathies are extremely rare in diabetic patients. Occulomotor nerve palsy presents with severe eye pain and paresis of extra ocular muscles innervated by it, accompanied by ptosis. It usually . It usually spares the pupil as the

parasympathetic fibres are in the periphery and the vascular origin in diabetes leads to centrofascicular involvement.

Diabetic Amyotrophy: This is usually seen in older patients and presents with unilateral or bilateral muscle weakness, severe pain and proximal atrophy of the thighs. It is thought to be due to immune mediated epineural microvasculitis, though the exact mechanism is not known.(12)

Diabetic Truncal Radiculoneuropathy:Truncal neuropathies are predominantly unilateral, with an abrupt onset and pain and dysesthesias as main features. They can sometimes be bilateral and involve the lower thoracic and abdominal wall in a girdle like distribution.

Acute Sensory Neuropathy: This is a distinctive variant of Diabetic peripheral neuropathy. This syndrome is also called **Diabetic cachexia**. Symptoms are usually severe pain, weight loss, cachexia, depression and sometimes erectile dysfunction in males. Clinical signs are rare with occasionally absent ankle reflex and allodynia. This can happen due to poor glycemic control or due to rapid improvement of glycaemia. The rapid changes in the blood glucose entry into the cells causing alterations in epineural blood flow, lead to ischemia(12). Pain fibres are predominantly involved. However the pathological basis of this condition is not yet determined, and immune mechanisms are likely(18). Natural history of the disease is resolution of symptoms in one year.

Insulin Neuritis:

This is a rare entity and is seen after starting the patients on insulin therapy. Studies have said that insulin causes a reduction in endoneurial oxygen tension in normal nerves, however diabetic nerves are resistant to these changes. However, once the hyperglycemia is controlled, the nerves become sensitive to insulin and can lead to neuritis(19). This could be immune mediated as well.

PATHOPHYSIOLOGY OF DIABETIC NEUROPATHY:

Diabetes affects the autonomic and peripheral nervous systemleading to diabetic neuropathy, the most common complication during the course of disease leading to increased mortality and morbidity. Diabetic neuropathy is a heterogeneous condition in view of its varied presentation which can be focal, multifocal or diffuse, proximal or distal. Multiple metabolic components and ischemic changes are responsible for diabetic neuropathy, most important being the hyperglycemia. Other factors responsible for DN are dyslipidemia, impaired insulin signaling and various other metabolic alterations as a result of above factors.

Hyperglycemia: Excess intracellular glucose influxvia different metabolic pathways leads to cell damage. Hyperglycaemia induces hypoxic environment and oxidative stress. These changes result in abnormal neuronal, axonal, and Schwann cell metabolism, which result in impaired axonal transport.Activation of protein

kinase C has been linked to vascular damage in DN.

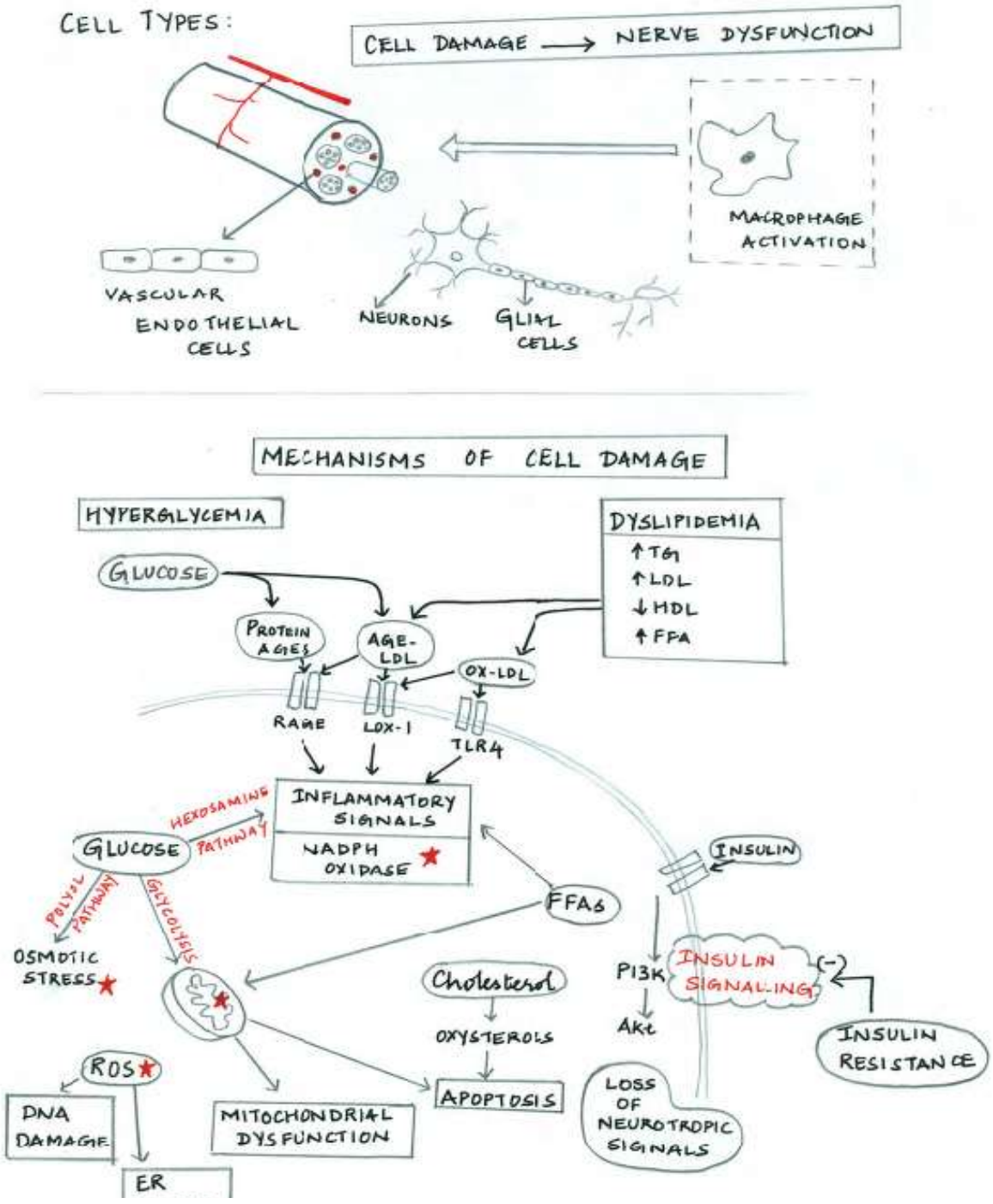


Figure 2: Pathophysiology of Diabetic peripheral neuropathy

Excessive glycolysis may overload mitochondrial electron transport which generates ROS. Influx through polyglycol pathway leads to intracellular hyperosmolarity, resulting in reduced NADPH levels and increased oxidative stress (Figure 2).Activation of Polyol pathway in the nerve through enzyme aldose reductase leads to accumulation of sorbitol and fructose in the nerve and induces non-enzymatic glycosylation of structural nerve proteins. Long term inflammatory signaling upregulates RAGE and activates NFkB. Increased glucose influx through the hexosamine pathway is associated with inflammatory injury.

Dyslipidemia: Dyslipidemia is found in many of the patients with Type 2 Diabetes and this also plays a role in the pathophysiology of diabetic neuropathy. Several underlying mechanisms have been identified. It has been observed in vitro that free fatty acids (FFAs) can directly cause injury to Schwann cells. They also have systemic effects such as promoting inflammatory cytokine release from adipocytes and macrophages. Plasma lipoproteins, particularly low-density lipoproteins (LDLs), can be modified by oxidation (oxLDL) and/or glycation, and these modified LDLs can bind to extracellular receptors (including the oxLDL receptor LOX153, Toll-like receptor 454 and RAGE47), triggering signaling cascades that activate NADPH oxidase and subsequently cause oxidative stress(Figure 2). Cholesterol may also be oxidized to oxysterols, which have a role in promoting apoptosis in neurons.

Impaired insulin signaling:

Insulin has been shown to have neurotrophic effects, promoting neuronal growth and survival. Insulin deficiency in Type 1 Diabetes and insulin resistance in type 2 diabetes cause a decrease in this neurotrophic signaling and probably contributes to the pathogenesis of diabetic neuropathy. As is seen in muscle and adipose tissue, in neurons also insulin resistance occurs by inhibition of the PI3K/Akt signaling pathway. Disruption of this pathway may also lead to mitochondrial dysfunction and oxidative stress, further promoting neuropathy. These mechanisms lead to multiple cellular disturbances, including mitochondrial dysfunction, endoplasmic reticulum (ER) stress, DNA damage and apoptosis.

SCREENING TESTS FOR DIABETIC PERIPHERAL NEUROPATHY:

Role of Michigan Neuropathy Screening Instrument:

Michigan Neuropathy Screening Instrument is a clinical tool for screening diabetic peripheral neuropathy. It comprises two parts, a self administered questionnaire which can be totalled up to arrive at a history score and an examination part, which can be performed easily by General practitioners and internists. It can be easily interpreted as well.

A study done by Ali Moghataderi et al had compared MNSI with NCS and obtained sensitivities and specificities for various cut offs. They observed that 79% sensitivity at a cut off value of >/= 1.5 decreases to 35% when the cut off is increased to 3. However the specificity increased with increase in the cut off value of MNSI.

It is a good screening tool, however, it is just a screening test and other methods are needed for confirming the diagnosis. (20)Another limitation of MNSI is its inadequacy for screening of the Autonomic nervous system.

Role of Semmes Weinstein Monofilament:

Back in the 1960s, a set of nylon filaments were first used by two neuropsychologists, Sidney Weinstein and Florence Semmes to assess the sensory loss in patients with penetrating brain injury. This came to be called Semmes Weinstein Monofilament. It had replaced the use of horse hair for sensory testing, overcoming a lot of the drawbacks of horse hair, one being absorption of humidity(21,22). Semmes Weinstein monofilament is a controlled instrument for sensory testing due to the fact that the nylon bends when an intended force of application is delivered. The monofilament is available in different sizes, eg. a monofilament with 5.07 gauze size delivers a 10 gram force and buckles when the 10 grams are delivered. This is called a 5.07/10 gram monofilament.

Semmes Weinstein monofilament is recommended as a screening tool for diabetic peripheral neuropathy by several guidelines. (23,24) It is a simple, inexpensive, easy to use and portable test and assesses loss of protective sensation.

Not only is it considered an effective screening tool for the outpatient departments, but also, patients who are willing to learn, can be taught how to use it, as it would help in early diagnosis and would motivate them for better glycemic control(25).

There is no standard method of application of monofilament. Some studies have recommended using it at one site (26) and others at several sites. There is difference in the interpretation of the test as well. A systematic review by Dros et al had included four studies in order to assess whether 10 gram monofilament was useful as a diagnostic test for peripheral neuropathy of any cause. The sensitivity of monofilament varied from 41 to 93% and the specificity varied from 68 to 100%. These differences are also possibly due to differences in study populations, differences in the methods of application and interpretation. They concluded that despite the frequent use of monofilament for screening of diabetic peripheral neuropathy, little can be said about its diagnostic value due to lack of studies with a standard technique and proper methodology. It cannot be used as a single diagnostic test and needs to be coupled with other clinical testing, and when in doubt, nerve conduction studies need to be done to establish the diagnosis. (27)

The sensitivity and specificity has widely varied between the studies(28–32). The reason for this could be that different studies have compared the Monofilament testing to different gold standards. Some studies have taken clinical testing as gold standard, while others have taken biothesiometer or thermal testing as gold standards. Other reasons for this could be that the test was applied in different populations and the method of application was different with differences in the sites and number of sites of testing. Diabetes can affect sensory nerves differently in different regions of the foot, and variation could occur due to testing over calluses also. (21)

Perkins et al compared the simple screening tests for peripheral neuropathy with the standard criteria of nerve conduction studies. The screening tools they used were Semmes Weinstein Monofilament, pin prick testing, vibration on-off method and vibration timed method (by a 128Hz tuning fork). Of the four sensory modalities, vibration testing by the on-off method had the highest positive likelihood ratio of 26.6 and a low negative likelihood ratio of 0.51. The specificity was 99% for five or more insensate responses. Both the10-g monofilament and superficial pain modalities had comparable likelihood ratios(10.2 and 9.2), but better sensitivity was observed with the 10-gmonofilament(40%) and better specificity was observed for superficial pain (97%), Semmes Weinstein monofilament, superficial pain sensation testing, and vibration testing by the on-

off method each required less than 60 seconds to perform accurately. Vibration testing by the timed method took longer depending on the degree of normalcy.(33)

Role of Biothesiometry in Diabetic Peripheral Neuropathy:

Biothesiometer is a device that is used to measure accurately the threshold of perception of vibration sense. Tuning fork with a frequency of 128 Hz has been widely used as a screening tool for diabetic peripheral neuropathy. Biothesiometer works as an electrical tuning fork and helps detect large fibre neuropathy earlier. It has a vibrating probe, which is placed on the patient's foot. The vibration amplitude, measured in volts can be increased gradually by turning a dial. The patient is asked to indicate as soon as the vibration is felt. The value is then recorded. In this way, the biothesiometer helps to detect the severity of neuropathy.

As in the case of any other device, biothesiometer also has its own limitations. There could be a confounding effect of the pressure applied on the vibrating probe, limb temperature, limb site, tactile surface of the skin, the understanding level of the patient and psychological factors. Despite all these disadvantages, it is considered as a good screening tool for diabetic neuropathy.

There is a controversy about the sensitivity and specificity of biothesiometer, which exists because of the difference in the gold standard tool used to determine the sensitivity and specificity of biothesiometer. Young et al has shown that the

sensitivity is 80% and specificity is 98%. This was a one year follow up study to observe the development of ulcer based on the vibration proprioception threshold value.(34) In the study by Pourhamidi et al, it was seen that the sensitivity was 82% and specificity was 70% for diabetic peripheral neuropathy (DPN) and was much lower for detection of small fibre neuropathy. Here the gold standard used for DPN was an abnormal NCS and Diabetic neuropathy Symptom Score of more than two. The gold standard for small fibre neuropathy was normal NCS, and abnormal thermal testing and Diabetic neuropathy Symptom score of more than 2. (29)

Armstrong et al used the presence of ulcer as a gold standard. This is the most reliable gold standard tool among all the available ones as the diseased and not diseased are clearly evident. According to this study, the sensitivity of biothesiometry was 95% and specificity was 65%. By combining other screening tools, such as Semmes Weinstein monofilament, or a neuropathy questionnaire with biothesiometer, there was a definite increase in specificity with a little or no decrease in sensitivity. (31)

Some studies have taken VPT (Vibration perception threshold) as the gold standard for diagnosis of peripheral neuropathy. However there is a difference in the cut off to define neuropathy. While certain studies have used 15 as the cut off (34,35) ,others have used 25 volt as cut off.(32) Some people have graded the severity based on the value of VPT. (36)

Young et al has shown that the cumulative incidence of foot ulcers in patients with a VPT of <15V is 2.9% and that of patients with a VPT of > 25V is 19.8% indicating a seven times increased risk of ulceration when the VPT is more than 25V when compared to a VPT of less than 15V. (34) This means that the patients with a VPT of more than 25V have to be explained the higher risk of ulceration and taught adequate foot care practices. The patients with biothesiometry values between 15V and 25V need to be advised strict glycemic control to at least delay the progression of peripheral neuropathy. This group of patients should be under regular follow up as well.

Studies have shown that age plays a significant role in reduction of vibration perception. However there is not much effect of gender. There is no right left variation for vibration perception. (36)

Dipa Saha et al conducted a study to assess if VPT testing can be applied in our country to diagnose diabetic peripheral neuropathy early(37). They had taken 60 diabetic patients, 30 with clinical evidence of neuropathy and 30 without clinical evidence of neuropathy based on Michigan Neuropathy screening instrument. In the group with clinical neuropathy, 26.6% had no neuropathy based on VPT using biothesiometer. This could be attributed to the fact that biothesiometry is a subjective test. Majority of the patients with clinical evidence of neuropathy had grade 2 severity according to biothesiometry. 60% of the patients without clinical evidence of neuropathy had grade 1 severity of neuropathy according to

biothesiometry. This shows that VPT testing using biothesiometer can pick up sub clinical cases of peripheral neuropathy and this could help in early institution of therapy, better glycemic control and prevention of disease progression.

Role of Nerve Conduction Studies:

Nerve conduction studies are considered the most reliable, accurate, sensitive, specific and validated diagnostic test to assess peripheral nerve function.(39,40) They are objective and non invasive tests which have long been considered minimal criteria or the gold standard for diagnosis of neuropathy(41). NCS is done for both motor and sensory nerves.

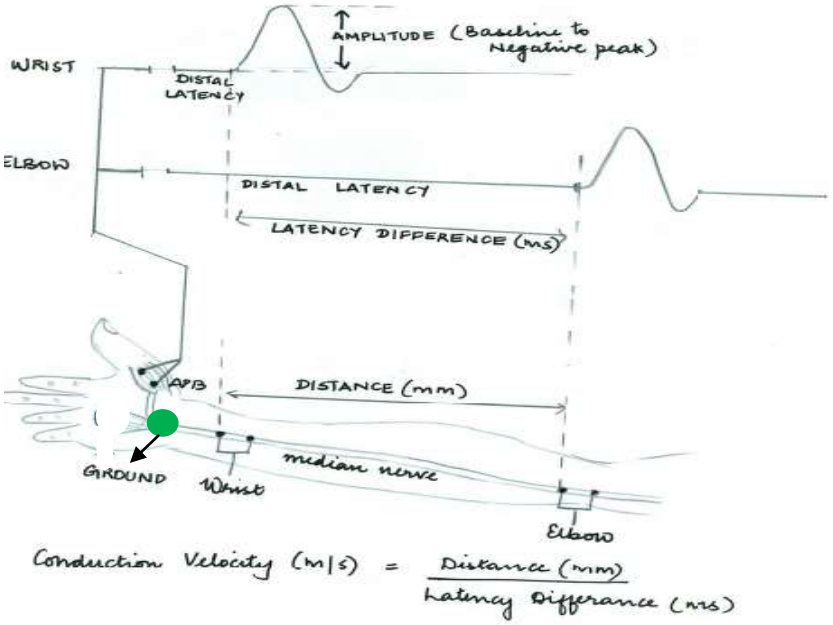


Figure 3: Measurement of Compound Motor Action Potential (CMAP)

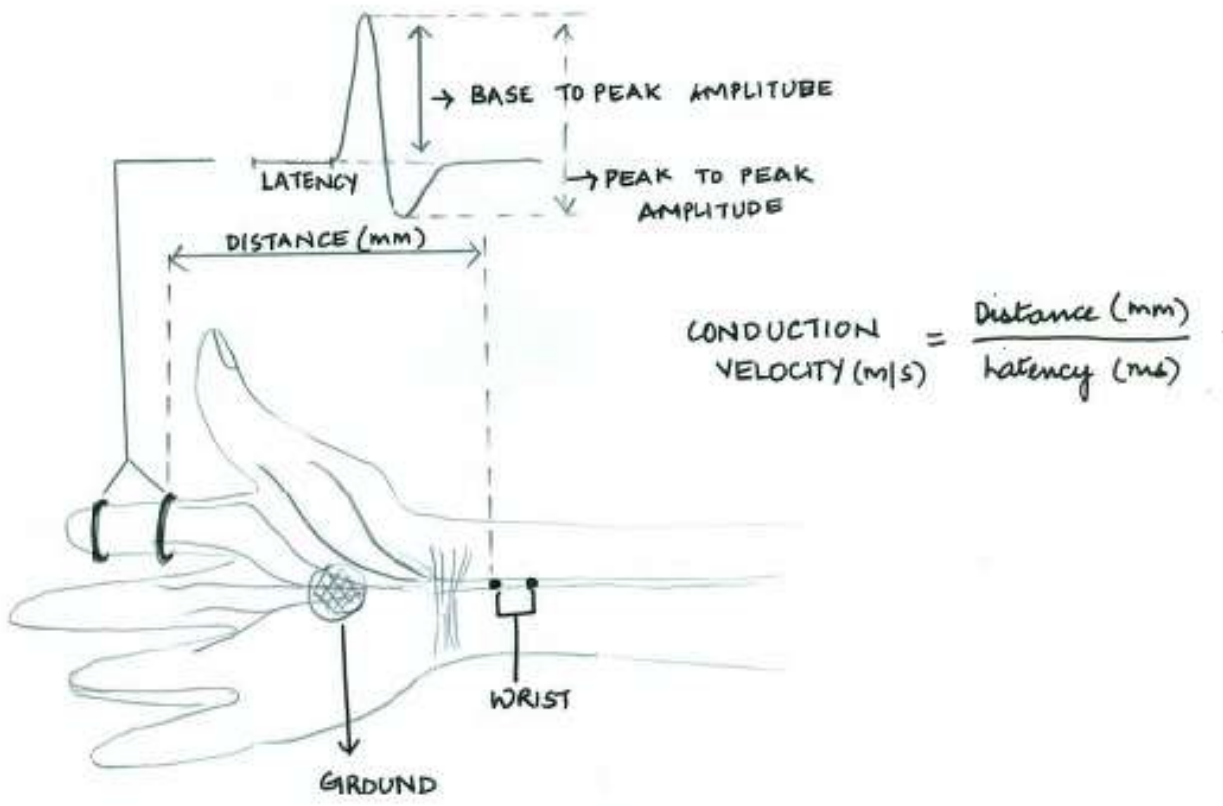


Figure 4: Measurement of Sensory Nerve Action Potential (SNAP)

For motor nerves, the stimulation is done in an orthodromic direction and a compound motor action potential is obtained. (Figure 4). For sensory nerves, the electrical stimulation is applied in the antidromic direction and a sensory nerve action potential is obtained(Figure 5).The nerves usually tested are radial, median and ulnar sensory and motor nerves of the upper limb and sural, superficial peroneal sensory and tibial and common peroneal motor nerves of the lower limbs. The parameters looked into are distal latency, amplitude and conduction velocity. However, it is important to decide how many nerves, which nerves and which parameters to assess. When we take all these parameters of so many different nerves**, the next question that arises is how to interpret the data**

and how to come to a conclusion whether the patient has diabetic neuropathy or not. The American Academy of Neurology(AAN) and PMR and Electrophysiology came to a consensus and their criteria said that when any two attributes of any two nerves, one being the sural nerve is affected, then a diagnosis of diabetic peripheral neuropathy can be made.(42)

In diabetic neuropathy, sensory, motor and autonomic involvement is seen. Motor and sensory abnormalities can be picked up by NCS with sensory nerves being affected more than motor nerves, however autonomic neuropathy gets missed. Of the large and small nerve fibres, myelinated and unmyelinated fibres, nerve conduction studies mainly assess the large myelinated fibres.

It is well known that the most common form of diabetic peripheral neuropathy is distal symmetric sensorimotor polyneuropathy which is length dependent and is predominantly sensory. As the severity of the disease increases, there is progressive involvement of motor fibres as well. An experimental animal modal study done on Streptazocin induced experimental diabetes, in mice with a duration of diabetes being 8 months, showed that in motor neurons, there were progressive features of distal loss of axonal terminals but there was no perikaryal dropout, indicating distal axon retraction. As the cell bodies in the axons are preserved, there is more of conduction velocity slowing and eventually loss of motor neurons with single motor unit action potential enlargement. There is a subsequent decrease in amplitude. This suggests that when compared to sensory neurons, motor neurons are resistant to the effects of diabetes, however they are eventually targeted by diabetes and undergo degeneration.(43)

As the progression of diabetic neuropathy is centripetal, the involvement of the most distal muscles occurs first and then the disease process advances proximally. A study was done on the axonal dysfunction in diabetic peripheral neuropathy, on 40 patients where the motor unit potentials were recorded from the Extensor Digitorum Brevis and were compared with the motor nerve conduction velocity and distal motor latency of the lateral popliteal nerve or the common peroneal nerve. Classical feature of diabetic neuropathy is axonal dysfunction with concomitant collateral reinnervation which parallels demyelinating lesions. The collateral reinnervation is one explanation to subclinical neuropathy being picked up by nerve conduction studies. In this study, they have observed that the fastest motor nerve conduction velocity is affected in diabetics with clinical neuropathy more than in those without. This has a positive correlation with the motor unit numbers as well. There is a negative correlation with age and duration of diabetes, which indicates that the higher the age and the higher the duration if diabetes, the conduction velocity and motor unit numbers are significantly affected.(44)

Some limitations have been identified with EDB, that is, since it is an intrinsic foot muscle, it would be difficult to differentiate axonal loss due to trauma from axonal loss due to the biochemical changes in diabetes. Keeping in view the centripetal progression, the next muscle to be affected would be Tibialis Anterior. Hence one study was done to investigate the motor unit loss in Tibialis Anterior. Another advantage quoted was that more loss of motor units is expected in a more functional muscle like Tibialis Anterior. They showed that there was 40% decrease in the CMAP amplitude, 50% increased single

motor unit action potential, signifying reinnervation and 60% decrease in motor units, indicating that the denervation is outpacing the collateral reinnervation.

The normative data for Nerve conduction tests must be standardized for every particular population as they vary very much with the ethnicity. They also have to be adjusted for age, height and gender. There are studies showing that there is a significant negative correlation for amplitude and conduction velocity, with age and height. (45,46) The fact that various factors affect the rate of nerve conduction make it a weak measure in the prediction of severity of peripheral neuropathy. Nerve conduction studies require specialized equipment and need expertise to perform. They are time consuming and complex. Technical errors can occur in patients with obesity.

Despite all the limitations in the applicability of NCS, it is a reproducible, objective and convenient measure for early detection of diabetic neuropathy and prediction of relevant late stage complications. It has also been found to correlate with the morphological findings of nerve biopsy. (47) NCS definitely have an important role in early detection and prediction of diabetic neuropathy, before clinical presentation. Hence they are fundamentally the most widely accepted test for diagnosis of diabetic peripheral neuropathy.(39)

In 1994, Feldman et al said that NCS alone was not enough for diagnosis of DPN, it had to be combined with clinical testing and this was called the MDNS (Michigan Diabetic Neuropathy Score)(48) . The 1998 San Antonio Consensus Statement said that multiple assessments including evaluation of symptoms, eliciting clinical signs, electrodiagnostic studies, Quantitative sensory testing and autonomic function testing are needed for proper

diagnosis and classification of Diabetic neuropathy.(49) However, the AAN criteria suggested that patients with abnormal NCS had a relatively high likelihood of the condition. It has recently been proposed that any NCS abnormality with signs and symptoms confirm the diagnosis of Diabetic peripheral neuropathy, abnormal NCS without clinical signs and symptoms are suggestive of subclinical neuropathy, signs and symptoms without an abnormal NCS are suggestive clinical or small fibre neuropathy.(50,51) Pourhamidi et al showed that in the impaired glucose tolerance population, there is a higher prevalence of small fibre neuropathy(32%) than distal symmetric peripheral neuropathy(12%), whereas in the group with Type 2 DM, the prevalence of small fibre neuropathy(28%) was similar to that of distal symmetric peripheral neuropathy(30%). (29)

Role of Sural Radial Amplitude Ratio (SRAR):

Sural Radial Amplitude ratio (SRAR) is calculated by dividing sural sensory amplitude and radial sensory amplitude. As axonal polyneuropathy is characterized by distal degeneration of neurons, and the disease process is a length dependent one, it is expected that the sural radial amplitude ratio would be one of the earliest parameters to be affected. Hence this is considered a useful test in detecting diabetic polyneuropathy.(52) However, there are inconsistent results in literature, giving rise to doubts about its reliability and usefulness.(53,54)

Again, there is no standard cut off for defining neuropathy by SRAR. One study had shown that a cut off of 0.4 had a high sensitivity and specificity (55), another study had

shown that a cut off of 0.34 was highly sensitive and specific(56), while another study has shown that majority of the normal persons have an SRAR of more than 0.21.(57)

Rutvoke et al conducted a study among patients with a diagnosis of polyneuropathy based on clinical and electrophysiological criteria. Patients were included if they had at least two of the four parameters abnormal; including reduced vibratory sense below the knees, reduced pin prick and light touch distally in the legs, markedly reduced ankle reflexes or a distal to proximal gradient of chronic reinnervation and/or ongoing denervation on EMG in the leg. Thirty patients and 30 age matched controls were included in the study. Of the 30 cases, 10 of them had diabetes mellitus, whereas the others had other reasons for polyneuropathy including alcoholism, late stage HIV, Renal failure, vasculitis, Crohn's disease, chemotherapy and unknown causes. A cut off of 0.4 for SRAR was used and any value less than 0.4 was considered as abnormal. The sensitivity and specificity of SRAR was found to be 90%, much better than an individual sural SNAP amplitude which had a sensitivity of 66% and specificity of 93%. SRAR was not influenced by age, although sural amplitude was influenced by age. This eliminates the need for age based normative values and is hence more useful and convenient. This could be because the amount of influence of age on sural nerve as well as radial nerve is the same, hence the overall influence on SRAR was not significant. This suggests that the reduction in sensory amplitude due to increasing age is in part due to nerve loss, at the dorsal root ganglion, rather than only a length dependent process(55). The finding that it is not influenced by age is supported by another study in normal subjects by Overbeek et

al. It was also identified that there was no influence of gender, height or weight on SRAR. (57)

A study was done by Jung Bin Shin et al to assess the usefulness of minimal F wave latency and sural radial amplitude ratio (SRAR) in early diagnosis of diabetic peripheral neuropathy. They had selected diabetic patients with symptoms or signs of peripheral neuropathy and performed conventional NCV as well as minimal F wave latency and SRAR in all these patients. They found that minimal F wave latency was prolonged in 67% of the patients with a normal motor conduction velocity. Hence they concluded that minimal F wave latency is a more sensitive parameter than both conduction velocity of motor fibres and the amplitude of the compound motor action potential and therefore electrophysiological studies of diabetic patients must include F wave as a routine. However they observed a strong correlation of the increase in minimal F wave latency with that of slow conduction velocity. They also said that SRAR could be considered an additional sensory nerve conduction study, especially when sural sensor nerve conduction is not clearly diagnostic.(52)

A study was done by Barnet et al in 49 diabetic patients, all of whom were diagnosed to have polyneuropathy based on a Consensus criteria. Out of these patients, 45 of them had neuropathy based on TCNS (Toronto Clinical Neuropathy Score). SRAR was done in all the patients and it was found that only 39% of them had an abnormal SRAR, however 74% had a low sural amplitude. It was concluded that SRAR had no added advantage when compared to sural amplitude in picking up cases with peripheral neuropathy. The

reason for this was that a cut-off of SRAR less than 0.21 was taken for diagnosis of neuropathy. (54)

Papanasi et al studied the usefulness of sural sensory/radial motor amplitude ratio for the diagnosis of peripheral neuropathy in type 2 diabetic patients. They attempted to identify a potential new electrophysiological index that might correlate well with the standard NCS. Sural sensory amplitude/Radial motor amplitude ratio was the most useful diagnostic index, with 85% sensitivity, 71% specificity, 91% positive prognostic value, 59% negative prognostic value and the highest overall agreement. Low levels of this ratio were associated with a nearly eightfold increase in the risk for NCS neuropathy. However this simple parameter cannot replace the entire nerve conduction studies in the diagnosis of diabetic peripheral neuropathy. This ratio, with a high sensitivity and a moderately high specificity, appears promising and merits further evaluation.(58)

Some limitations of SRAR have been noted. It is a ratio of two separate nerves and hence any mild isolated neuropathy in either of the nerves can cause a big difference in the ratio. Technical precision is crucial for accurate values.

Role of minimal F wave latency:

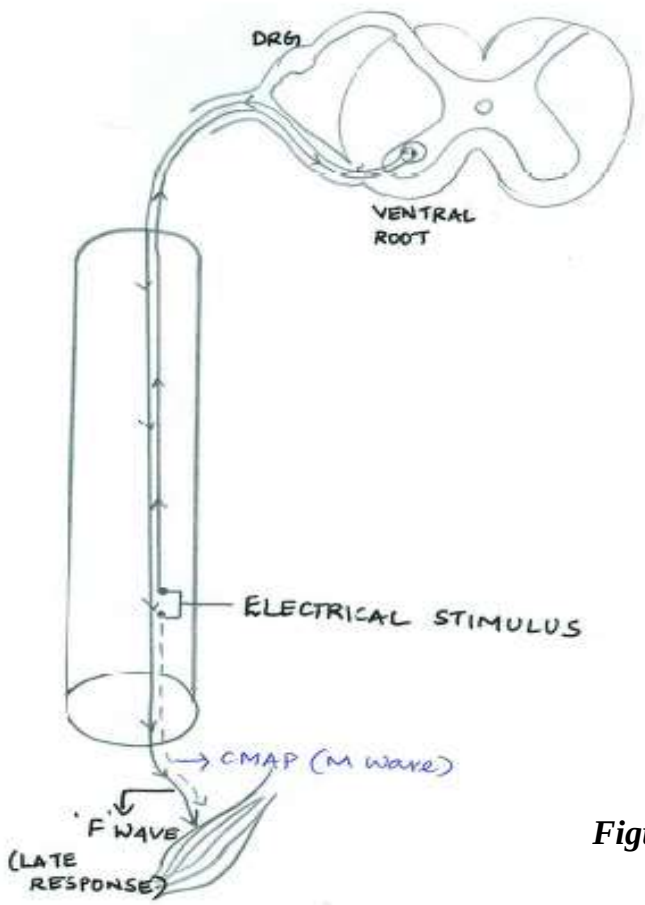


Figure 5: Physiology of F wave

F wave is a small late response, an antidromic motor response, occurring after the CMAP. It traverses the peripheral nervous system twice, once from the site of stimulation to the anterior horn cell, and then from the anterior horn cell back to the muscle innervated by the nerve stimulated(Figure 3). It evaluates the motor neurons and tells us about the excitability of the motor neuron pool. It was originally described by Magladery and Mc Dougal in the year 1950.(59) It is called F wave because it was first described in the foot muscles. However it is a ubiquitous response and can be recorded from all skeletal muscles. The F waves are characterized by variability in amplitude,

latency and configuration because different spinal motor neurons are stimulated with each stimulus. Hence at least 10-20 F waves have to be recorded with a supra maximal stimulus each time. The commonly used parameters include minimal F wave latency, mean F wave latency, maximum F wave latency, F wave dispersion or chronodispersion, F wave amplitude and F wave persistence.

F waves are clinically commonly used to evaluate proximal nerve lesions for example lumbosacral radiculopathy and Gullian Bare Syndrome. Since diabetic neuropathy is a condition where the distal segment is more severely and early involved, F wave was not routinely used for the diagnosis. However due to its long pathway, for a diffuse peripheral lesion, even in early stages it will be reflected. Studies have said that minimal F wave latency is a useful parameter in early diagnosis of diabetic peripheral neuropathy. (60–62) However, some other studies have contradicted this fact. (63)

A.R.Garate and A.G.Joshi conducted a study on utility of minimal F wave latency for diagnosis of peripheral neuropathy. They included 60 patients who were diagnosed with type 2 diabetes mellitus and had symptoms of peripheral neuropathy. Motor and sensory nerve conduction studies and F waves were performed in bilateral upper and lower limbs. It was found that the most sensitive parameter was minimal F wave latency. The changes in minimal F wave latency and distal motor latency ($p<0.005$) were more significant than the changes in the amplitude ($p<0.01$). This could be attributed to the fact that initially there is loss of myelin sheath which leads to an increase in latency. Only when the axonal loss happens, the muscle fibre mass decreases and hence there is a decrease in the CMAP

amplitude. They also found that in 20.41% of the motor nerves studied, F wave minimal latency was increased while other motor conduction parameters like distal motor latency, motor nerve conduction velocity and compound muscle action potential were normal. Another finding was that F wave latency was more affected in the upper limbs than in the lower limbs.(60)

Barathi Taksande et al studied the usefulness of F wave latency measurement in the diagnosis of diabetic polyneuropathy. They said that the minimum F wave latency had a larger Z score or standard score than the motor conduction velocity and CMAP (compound motor action potential) amplitude of the median, ulnar, peroneal or tibial nerves, thus implying that F wave latency was affected more than the standard NCS parameters. There was a significant correlation between the minimum F wave latency and the motor conduction velocity in all the four motor nerves. This is because the slowing of nerve conduction is maximized by F waves travelling for long distances over the entire length of the nerve. (64) These findings coincide with that of Shin et al. (52)

The big drawback is that when studies have compared F wave with conventional NCS parameters, they have not used any gold standard. Most of the studies have included patients with symptoms of polyneuropathy, however have not quantified the symptoms.(60,64) The sensitivity and specificity are calculated based on the presence or absence of symptoms. If the clinical outcome measure was the presence or absence of an ulcer, then it would be reliable. However, when it comes to symptoms, it may be very subjective and without a proper screening system it would be difficult to rely on. To identify whether it is more useful than conventional NCS there should be a tool better

than NCS that can be considered for comparison. However as such a tool doesn't exist, so the question arises, are we really picking up sub clinical cases or are we picking up false positive cases.

Taha S Ahmed et al conducted a study to assess the usefulness of F-wave and sural potential in the diagnosis of subclinical diabetic neuropathy in patients from Saudi Arabia. This study was different from previous studies in that diabetics without clinical signs and symptoms of neuropathy and normal subjects were the participants. They had shown that sural nerve sensory conduction velocity, sural SNAP amplitude, tibial and peroneal minimal F wave latency and F wave duration were significantly different between the two groups. Hence they concluded that minimal F wave latency and F wave duration of tibial and peroneal nerves were the first to be affected in sub-clinical peripheral neuropathy. (61)

JUSTIFICATION OF THE STUDY

From literature what we infer is that there is a wide difference in the prevalence of diabetic peripheral neuropathy in various studies. This could be attributed to many factors including the lack of proper diagnostic criteria, gold standard test used, awareness of the population and other confounding factors like duration of diabetes. But the most important reason of all this would be the lack of diagnostic criteria. For example some studies have used VPT using biothesiometry as the gold standard whereas some studies have used nerve conduction studies as the gold standard. If we take nerve conduction studies, there is no single universal criteria followed. Similarly if we take VPT testing using biothesiometer, some studies use 15microV as the cut-off, while others use only the value of more than 25microV to diagnose peripheral neuropathy. In this way sub-clinical cases with neuropathy could be missed.

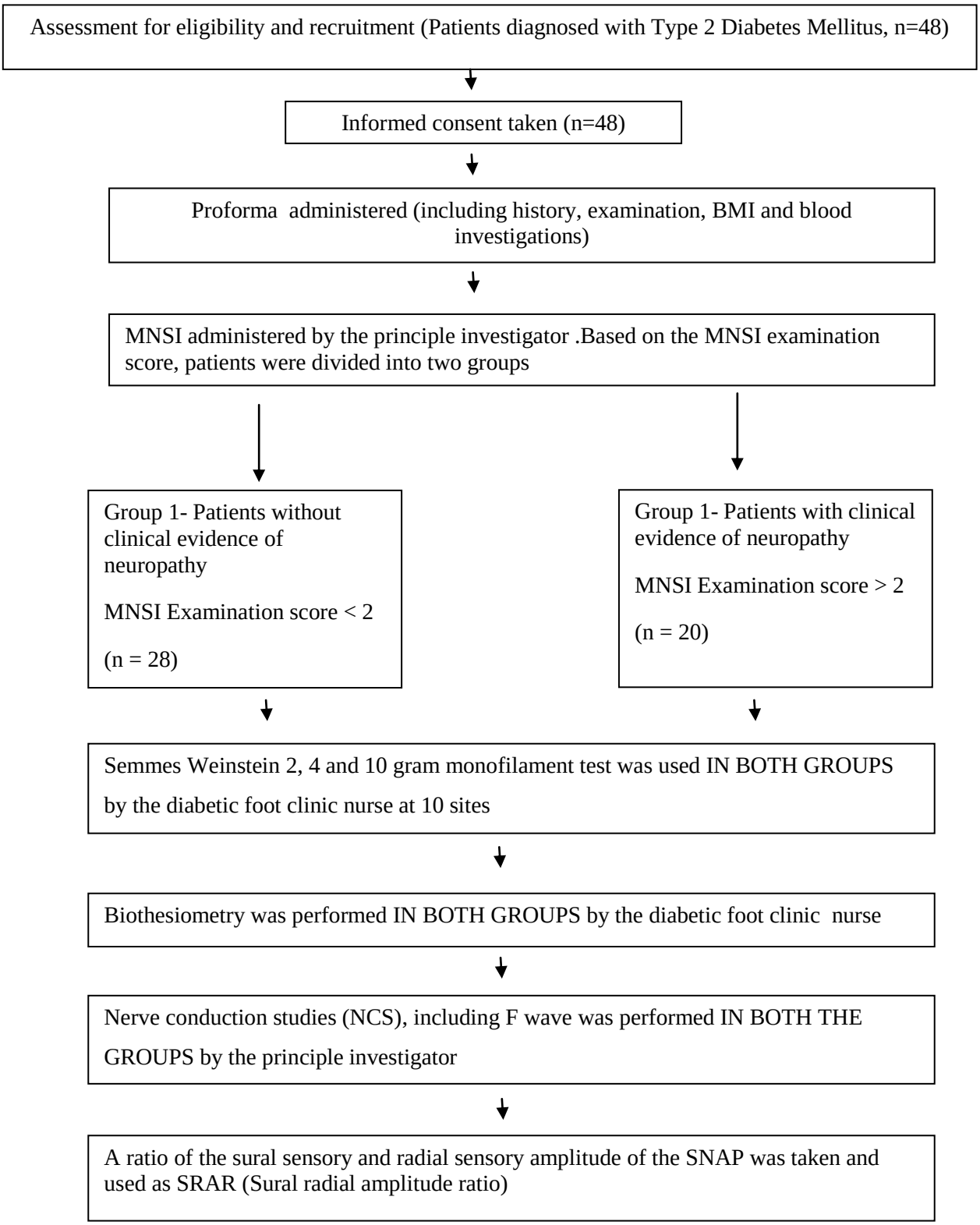
Despite all the controversy, many studies have considered NCS as the gold standard as it is an objective and reliable test. However they are time consuming and difficult to do. In the present study comparison will be made between various outpatient screening tools (Michingan Neuropathy Screening Instrument, biothesiometry, Semmes Weinstein Monofilament) and Nerve Conduction Studies. The diagnostic accuracy of each test when compared to NCS will be assessed. Biothesiometry is routinely being used in all patients presenting to the diabetic clinic. We would identify a few more simple tests to increase the sensitivity and specificity of diabetic neuropathy screening. We would also be assessing the usefulness of minimal F wave latency and sural radial amplitude ratio in early diagnosis of diabetic peripheral neuropathy.

SUBJECTS AND METHODS

This is a prospective cross-sectional study to compare the standard outpatient tools and nerve conduction studies in Diabetic peripheral neuropathy. The study was conducted in the Department of Physical Medicine and Rehabilitation. Forty eight patients with Type 2 Diabetes Mellitus, aged between 30-65 years, who met the inclusion and exclusion criteria were enrolled from June 2015 to June 2016 after getting informed consent. Patients were recruited from the Endocrinology OPD, Diabetic foot clinic and Physical Medicine and Rehabilitation OPD.

Baseline demographic parameters such as age, sex and duration of diabetes were assessed. A clinical proforma, which included a detailed history and examination was administered. Blood investigations, including fasting and post prandial sugars, HbA1C, Serum Creatinine and lipid profile were done. Michigan Neuropathy Screening instrument was administered and the patients were divided into two groups –Group 1 without clinical neuropathy and Group 2 with clinical neuropathy. There were 28 patients without clinical neuropathy and 20 patients with clinical neuropathy. Thereafter, monofilament testing, biothesiometer and nerve conduction studies were done in both groups.

Diagrammatic Algorithm



Participants:

Inclusion Criteria:

Patients between 30to 65 years of age diagnosed to have Type 2 diabetes mellitus

Exclusion Criteria:

1. Patients with ulcers/ amputations
2. Patients with other diseases which affect the peripheral nerve function like malnutrition, alcoholism, familial and chronic liver disease, chronic kidney disease.
3. Clinical evidence of any other peripheral nerve lesions/ lumbosacral radiculopathy/ lumbar canal stenosis
4. Patients with cardiac pacemaker/cardiac rhythm abnormalities.
5. Patients with Charcot foot
6. Patients with obesity (Absence of SNAPs in these patients could be due to technical errors)

The following tests were done.

1. MICHIGAN NEUROPATHY SCREENING INSTRUMENT:

PART 1 of this instrument is a self-administered Questionnaire.(Annexure 6) Responses of "yes" to items 1-3, 5-6, 8-9, 11-12, 14-15 are each counted as one point. A "no" response on items 7 and 13 counts as 1 point. Item 4 is a measure of impaired circulation and is not included in the score. Item 10 is a measure of

general asthenia and is not included in the score. ***A higher score (out of a maximum of 13 points) indicates more neuropathic symptoms***.

PART 2 Brief Physical Examination

This has the following components:

A. **Foot Inspection:**

Bothfeet were inspected for evidence ofdry skin, callous formation, fissures, infection and deformities such as flat feet, hammer toes, overlapping toes, hallux valgus, joint subluxation, prominent metatarsal heads, medial convexity (Charcot foot) and amputation.

Each foot with any abnormality receives a score of 1 for each side.

B. Presence or absence of ulcer:

Each foot with an ulcer receives a score of 1 for each side.

C. Assessment of vibration sense on the dorsum of the great toe:

This test was performed with the great toe unsupported. The test was done bilaterally - 128 Hz tuning fork was placed over the dorsum of the great toe on the bony prominence of the DIP joint. Normally, the examiner should be able to feel vibration in his or her hand for 5 seconds longer than a normal subject can at the great toe.

Scoring:

Present – if examiner sensed the vibration on his or her finger for < 10 seconds longer than the subject felt it in the great toe– scored as 0

Reduced – if examiner sensed the vibration on his or her finger for ≥ 10 seconds than the subject felt it in the great toe–scored as 0.5 for each side.

Absent – if no vibration was detected by the patient–scored as 1 for each side

D. **Grading of ankle reflex**:

Ankle reflex is elicited and if the reflex is absent the patient is asked to do the Jendrassic manoeuvre and if present , the reflex is designated as present with reinforcement.

Scoring:

Present - 0

Present with reinforcement - 0.5

Absent - 1

E. **Monofilament testing using Semmes Weinstein 10 g monofilament:**

The foot was kept supported. The filament was initially pre-stressed(4-6 perpendicular applications to the dorsum of the examiner's first finger).The monofilament was applied to the dorsum of the great toe midway between the nail fold and the DIP joint. The filament was applied perpendicularly and briefly, (<1 second) with an even pressure. When the filament bends, the force of 10 grams has been applied. The patient whose eyes were closed was asked to respond yes if he felt the filament.

Scoring:

Normal: Eight correct responses out of 10 –scored as 0

Reduced: One to seven correct responses–scored as 0.5

Absent: No correct answers –scored as 1

The total possible score of the part B of the Michigan Neuropathy Screening Instrument is 10. Patients were divided into two groups based on this test. A score of more than 2 was considered to be positive for neuropathy.

2. VIBRATION PERCEPTION TESTING USING A BIOTHESIOMETER:

The Biothesiometer was applied perpendicular to the test site with a constant and firm pressure. It **was performed using a Vibrometer-VPT machine, number V114012706 (Diabetic Foot Care India Private Limited)**

Figure 6a: A biothesiometer

Figure 6b: Vibration perception testing with the vibration probe over the first DIP joint

The vibration proprioception was *measured over the first DIP joint of both the legs*. The voltage was slowly increased at the rate of 1 mV/sec and the vibration perception testing value was defined as the voltage level when the subject indicated that he or she first felt the vibration sense.

The mean of three records was taken.

Scoring:

<15mV – normal

15-25mV – mild neuropathy

25-40mV – moderate neuropathy

>40mV - Severe neuropathy

3. SEMMES WEINSTEIN 2, 4 AND 10 GRAM MONOFILAMENT TESTING:

The foot was supported. Initially –pre-stress was done (4-6 perpendicular applications to the dorsum of the examiner's first finger). 2, 4 and 10 gram monofilaments were used.

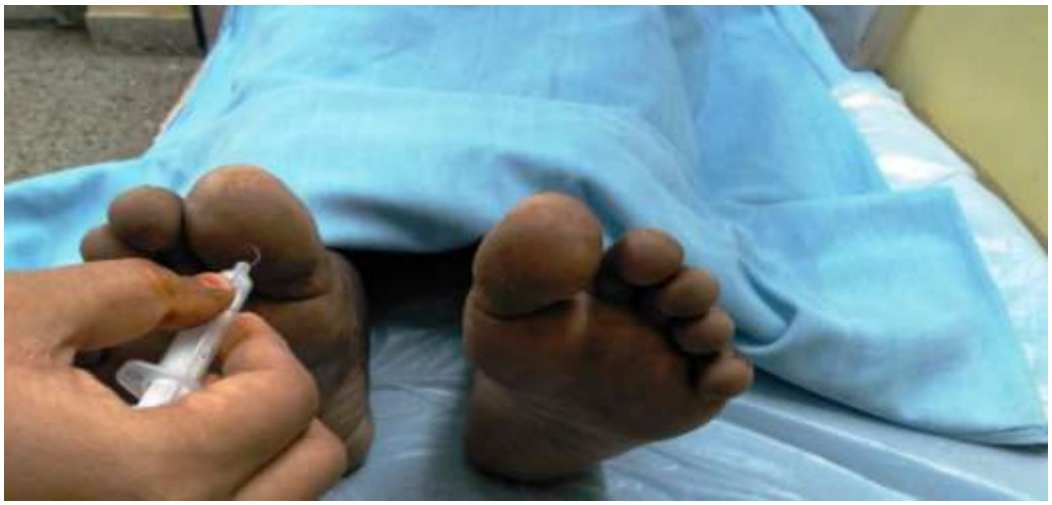


Figure 7: Semmes Weinstein Monofilament Testing

The filaments were applied to 10 sites including 9 plantar sites and 1 dorsal site. The plantar sites included the ventral aspect of digits 1,3 and 5; metatarsal heads (1,3,5), medial and lateral midfoot and heel. The dorsal site was the site between the base of digits 1 and 2. The filament was applied perpendicularly and briefly, (<1 second) with an even pressure. When the filament bends, the force of 2/4/10 grams has been applied(Figure 6). More than or equal to5 incorrect responses out of 10 in one foot indicated the presence of neuropathy.

4. NERVE CONDUCTION STUDIES:

Nerve conduction studies were performed on a Medelec synergy system (Multi sync LCD1770NX) with a room temperature of 23degrees. These studies were done using standard surface stimulating and recording techniques. Electrodes were coated with electro conductive gel and held in place with adhesive tape.

Figure 8:Performing the sural sensory nerve conduction study

The following studies were done:

i. Motor NCV was measured by electrical stimulation of a peripheral nerve and recorded from a muscle supplied by the nerve. The time taken for the electrical impulse to travel from the stimulation to the recording site was measured as the latency measured in milliseconds (ms). By stimulating in two different locations along the same nerve, the NCV(conduction velocity) across different segments could be determined. Calculations were performed by dividing the distance between the proximal and distal sites of stimulation by the differences in latencies (ms) to obtain nerve conduction velocity (m/s). The compound motor action potential amplitude (CMAP) amplitude was also measured. This was done for median, ulnar, tibial and common peroneal nerves

ii. Sensory NCV was measured by electrical stimulation of a peripheral nerve and recording from a purely sensory portion of the nerve, such as on a finger. Like the motor studies, sensory latencies are on the scale of milliseconds. The sensory NCV was calculated based upon the latency and the distance between the stimulating and recording electrodes. SNAP Amplitude of Sural, Superficial peroneal, radial and median nerves was also measured.

iii. Minimal F wave latencies of tibial, peroneal, median and ulnar nerves were recorded using a supramaximal stimulus with antidromic stimulation.

iv. Sural radial Amplitude ratio (SRAR) was calculated by dividing the SNAP amplitudes of sural and radial nerves.

**Nerve conduction studies were done in 25 normal subjects. Data was analysed. The mean and standard deviation was calculated for every parameter. Mean + 2SD

was taken as the cut off for latency and mean - 2SD was taken as the cut off for amplitude and conduction velocity. Based on this it was determined whether each parameter was normal or abnormal.

**For SRAR (Sural Radial Amplitude Ratio) >0.4 was considered as normal. (55)

**For minimal F wave latency normal values were taken from an Indian study, done in Gujarat, in 59 subjects and published in 2013. (59)

***According to the American Academy of Neurology, the American Association of Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation, there were many previous recommendations regarding NCS criteria for the diagnosis of polyneuropathy, but no formal consensus existed.

The following recommendation based on electrophysiologic principles combine both the highest sensitivity and specificity as well as the highest efficiency for the diagnosis of distal symmetric polyneuropathy. Hence the following recommended protocol for nerve conduction studies was used to determine the presence or absence of neuropathy.

This protocol included *unilateral studies of sural sensory, ulnar sensory, and median sensory nerves, and peroneal, tibial, median, and ulnar motor nerves with F waves*. **The minimum case definition criterion for electrodiagnostic confirmation of distal symmetric polyneuropathy is an abnormality (99th or 1st percentile) of any attribute of nerve conduction in two separate nerves, one of which must be the sural nerve**.(42)

Nerve conduction studies were done by the principal investigator under the supervision of PMR consultants who are co-investigators in the study. The entire procedure of NCS is mentioned in Annexure 7.

OUTCOME MEASURES:

1. CMAP amplitude, latency and conduction velocity of tibial, peroneal, median and ulnar nerves

2. SNAP amplitude, latency and conduction velocity of radial, median, ulnar, sural and superficial peroneal nerves

3.Minimal F wave latency of tibial, peroneal, median and ulnar nerves

4. SRAR (sural radial amplitude ratio)

5. Presence or absence of neuropathy based on the minimum voltage at which the patient first felt vibration sense while testing with a biothesiometer

6. Presence or absence of neuropathy based on the number of points perceived by a 2, 4 and 10 gram Semmes Weinstein Monofilament.

7. Presence or absence of neuropathy based on Michigan Neuropathy Screening Instrument examination score.

8. Michigan neuropathy screening instrument history score.

9. Blood investigation to assess glycemic status and nephropathy - Haemoglobin, Glycosylated Haemoglobin, fasting plasma glucose , Post prandial blood glucose, Serum Creatinine, Fasting serum lipid profile, Urine microalbumin.

Predictors of diabetic peripheral neuropathy:

1. Glycemic control(HbA1c, fasting blood glucose and post prandial blood glucose)

2. Duration of Diabetes

Confounding factors:

1. Age
2. Gender
3. Diabetes duration
4. Current smoking
5. Systolic blood pressure
6. Waist circumference
7. Height
8. Peripheral arterial occlusive disease
9. Glycosylated hemoglobin
10. Estimated glomerular filtration rate
11. Lipid profile.
12. Microalbuminuria

Statistical Analysis:

a. Sample size

Two Means - Hypothesis testing for two means	
	SURAL_SENSORY
Standard deviation in group I	3.93
Standard deviation in group II	6.76
Mean difference	3.41
Effect size	0.637979
Alpha error (%)	5
Power (1- beta) %	80
1 or 2 sided	2
Required sample size per group	41

With reference to Diabetics and Metabolic syndrome: Clinical research and reviews 8(2014)48-52. Table 5 the NCV results for Sensory Sural nerve of the Left leg was found to be 55.55±3.93 and 52.14±6.76 for patient without clinical neuropathy and with clinical neuropathy respectively. With alpha error at 5% and power at 80% for a two sided test we need to study ***41 patients with clinical evidence of diabetic peripheral neuropathy and 41 patients without clinical evidence of diabetic peripheral neuropathy.***

Formula

$$n = \frac{2s_p{}^2\left[Z_{1-\alpha/2} + Z_{1-\beta}\right]^2}{\mu_d^2}$$

$$s_p^2 = \frac{s_1^2 + s_2^2}{2}$$

Where,

S_1^2 : Standard deviation in the first group

S_2^2 : Standard deviation in the second group

μ_d^2 : Mean difference between the samples

α : Significance level

1- β : Power

Data entry was done using MS excel. Data was summarized using mean(SD) for normal data, median(range) for skewed data, for continuous variables and frequency(percentage) for categorical data.

Data was analysed using STATA/IC 13.1.

The mean (SD) of latency, amplitude and conduction velocity of normal nerves were calculated and mean +/- 2SD was used to divide the values into normal and abnormal. The normal and abnormal NCS (nerve conduction studies) groups were taken as outcome and further analysis was done.

The baseline characteristics among the clinical neuropathy and the clinically silent group were compared using Independent T test/ Ranksum test for continuous variables and chi square test for categorical variables.

The associations between MNSI and other categorical variables as well as NCS and other categorical variables were analysed using Chi Square test.

The diagnostic accuracies(sensitivity, specificity, positive predictive value and negative predictive value) were calculated comparing the NCS normal and abnormal group, taking NCS as gold standard. Kappa statistics was used to check the agreement between NCS and biothesiometer, and NCS and MNSI (Michigan Neuropathy Screening Instrument).

Correlation between amplitude and conduction velocity was analysed using Pearson correlation coefficient and scatter plots were presented.

Comparison between Neuropathy based on NCS and Age, gender, duration of diabetes, BMI, HbA1C and MNSI History Score were done using Independent T test and Ranksum test to explore the relation.

A multivariate logistic adjustability for age and HbA1C with NCS as the gold standard was analysed to study the risk of influence of age and HbA1C over the presence of neuropathy by NCS.

The initial sample size calculated was 41 per group (without and with clinical neuropathy). However after reaching a sample size of 28 in the group without clinical neuropathy and 20 in the group with clinical neuropathy the significance and power of the study were looked into. For sural conduction velocity, a significance with a p value of 0.0139 was achieved, however the power was less than 80%. For Sural amplitude the p

value was 0.0003 and the power was 91%. There was a good correlation between sural amplitude and conduction velocity. This was a pilot study and it was stopped at this point. We plan to continue the study after submission of thesis to attain a better sample size.

RESULTS:

During the study period of 1 year, 48 patients with type 2 Diabetes who satisfied the exclusion and inclusion criteria were recruited. Michigan Neuropathy Screening Instrument (MNSI), biothesiometry, Semmes Weinstein Instrument screening and Nerve conduction studies including F wave studies and Sural Radial amplitude ratio were done for all the patients.

1. BASELINE CHARACTERISTICS:

Of the total 48 patients, 36 were males and 12 were females. The mean age was 51.31 years and the mean duration of diabetes was 5.95 years. The mean BMI was 24.95kg/m^2 and mean HbA1C was 8.21. All the patients recruited were on treatment for diabetes mellitus with either insulin or OHAs.Based on the scores of MNSI, the patients were divided into 2 groups- Group 1 with no evidence of clinical neuropathy and Group 2 with evidence of clinical neuropathy i.e. MNSI examination score of >/=2.

Table 1: Baseline Characteristics:

	Group 1 **Mean(SD)**	Group 2 **Mean(SD)**	P value
Age	48.18(6.76)	55.7(7.39)	0.00065
BMI	24.32(3.01)	25.82(2.81)	0.08
HbA1C	8.28(2.15)	8.1(1.73)	0.756
Duration of Diabetes Median (Min,Max)	4(0.2,15)	6(0.2,20)	0.39

BMI - Body Mass Index, HbA1C - glycosylated haemoglobin, MNSI - Michigan Neuropathy Screening Instrument, SD - Standard Deviation

The above table shows that the mean age of patients without clinical neuropathy was 48.18(6.76) and the mean age of patients with clinical neuropathy was 55.7(7.39) and the difference was statistically significant (p=0.00065). The median duration of diabetes was 4 years (0.2,15) in the group without clinical neuropathy and 6 years (0.2,20) in the group with clinical neuropathy and this difference was not statistically significant. The mean HbA1C was 8.28(2.15) and 8.1(1.73) in the patients without clinical neuropathy and those with clinical evidence of neuropathy. This difference was not statistically different. The mean BMI (body mass index) in the patients with and without clinical neuropathy was 25.82(2.81) and 24.32(3.01) respectively.

2. DIAGNOSIS OF DIABETIC PERIPHERAL NEUROPATHY

2.1 Diagnosis of diabetic neuropathy based on MNSI

Based on Michigan Neuropathy Screening Instrument, 58.33% patients had diabetic peripheral neuropathy .

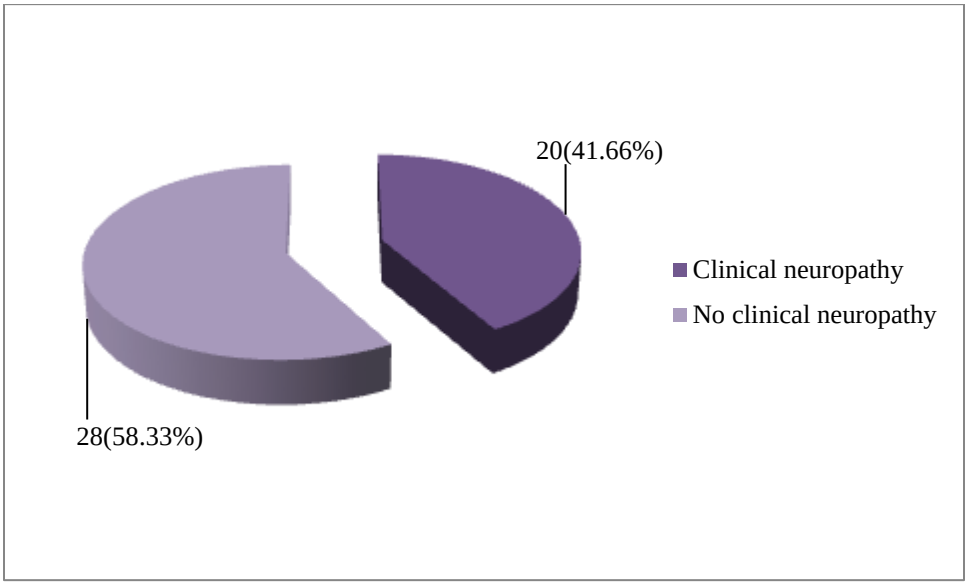


Fig 2.1. Diagnosis of Diabetic Neuropathy based on Michigan Neuropathy Screening Instrument(Clinical Neuropathy)

2.2 Diagnosis of Diabetic Neuropathy based on Biothesiometry:

According to biothesiometry, with a 15 mV cut off, 43.75% of patients had no neuropathy, 37.5% had mild neuropathy, 6.25% had moderate neuropathy and 12.5% had severe neuropathy. Thus a total of 56.25% patients were found to have neuropathy based on biothesiometry.

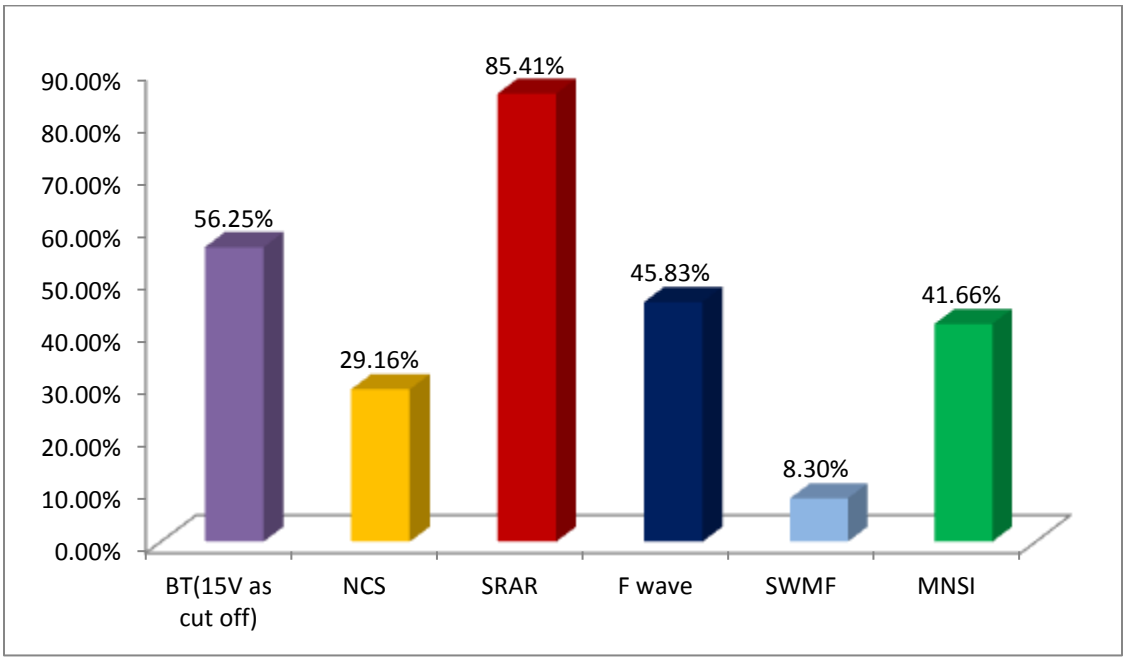
Table 2.2 Diagnosis of Diabetic Neuropathy based on Biothesiometry

Biothesiometer	Number(Percentage)
Normal	21(43.75%)
Minimal	18(37.5%)
Moderate	3(6.25%)
Severe	6(12.5%)

2.3. Diagnosis of Diabetic Neuropathy based on nerve conduction studies:

With the nerve conduction studies, only 14/48 (29.16%) were found to have neuropathy. Twenty seven percent of the patients had abnormalities in conduction velocity and 20% had abnormality in amplitude. An abnormal sural radial amplitude ratio (a computed ratio from nerve conduction studies) was noticed in 85.41% patients and abnormal F wave was seen in 45.83% of patient.

2.4 Diagnosis of Diabetic Neuropathy based on various screening tools:



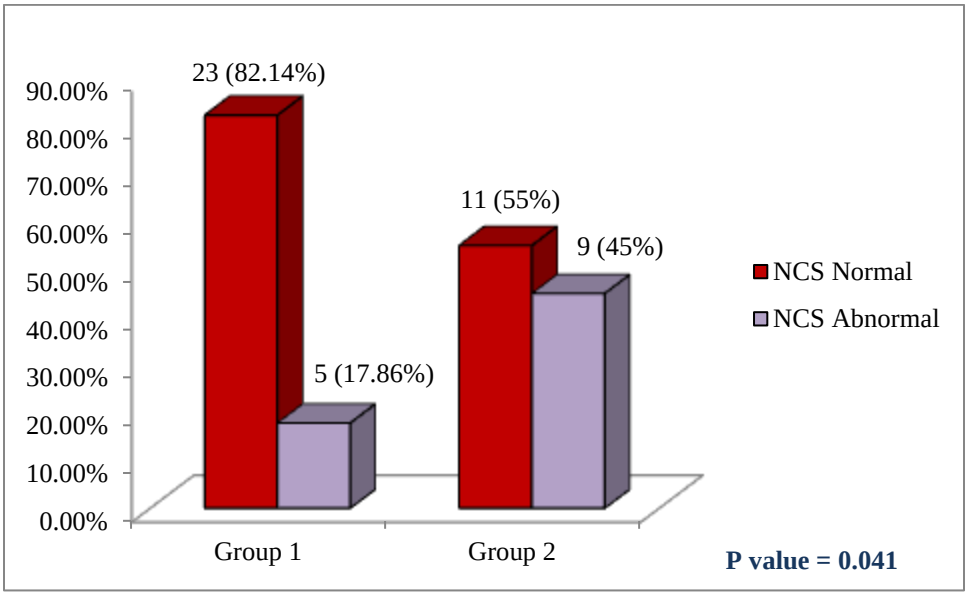
BT - Biothesiometer; NCS - Nerve Conduction Studies; SRAR - Sural Radial Amplitude Ratio, SWMF - Semmes Weinstein Monofilament; MNSI - Michigan Neuropathy Screening Instrument

Fig 2.4Diagnosis of Diabetic Peripheral Neuropathy according to various screenina tools

The above figure shows that 29.16% of the patients are diagnosed with neuropathy based on nerve conduction studies, 56.25% of the patients are diagnosed based on biothesiometer with 15V as cut off, 8.3% based on Semmes Weinstein Monofilament, 41.66% based on MNSI, 85.41% based on SRAR and 45.83% based on F wave.

3. COMPARISON OF VARIOUS SCREENING TOOLS FOR DETECTION OF DIABETIC PERIPHERAL NEUROPATHY

3.1 Relation between Clinical Neuropathy based on MNSI and Nerve conduction studies:



*NCS - Nerve conduction studies

Fig 3.1. Clinical Neuropathy based on Michigan Neuropathy Screening Instrument and Nerve conduction studies

This bar graph shows that in the group with no clinical evidence of neuropathy, 82.14% also have normal nerve conduction studies(NCS), whereas 17.8% have been diagnosed to have clinically silent neuropathy by NCS. However in the group with clinical neuropathy, 45% are diagnosed by NCS, the rest 55% have normal NCS. The correlation was significant with a p value of 0.041.Of the 14 patients with an abnormal NCS, 9 patients (64.28%) had clinical evidence of neuropathy.

The agreement between MNSI and NCS was 66.67% with a kappa of 28.36

Table 3.1.1. Comparison of CMAP/SNAP conduction velocity between the two groups (with and without clinical neuropathy):

	Right			Left		
	Group 1	Group 2	P value	Group 1	Group 2	P value
Sensory:						
Median	52.53(8.47)	48.96(12.65)	0.25	53.78(7.22)	48.46(5.87)	0.0092
Radial	61.44(7.32)	62.63(10.77)	0.65	61.08(7.98)	60.02(11.26)	0.70
Sural	47.15(15.06)	30.38(26.22)	0.007	46.49(10.95)	38.45(20.59)	0.09
Superficial peroneal	45.36(24.69)	25.52(27.81)	0.012	44.49(20.03)	36.54(26.86)	0.25
Motor:						
Median	53.11(4.9)	47.67(10.91)	0.024	52.14(6.26)	51.45(5.13)	0.68
Ulnar	57.45(6.87)	54.52(6.45)	0.14	56.17(7.74)	54.96(6.10)	0.56
Common peroneal	44.29(4.56)	40.98(7.31)	0.18	43.68(5.54)	41.07(7.77)	0.18
Tibial	43.28(5.94)	39.51(5.34)	0.028	44.30(4.53)	40.16(5.75)	0.0075

The above table shows that there is a significant difference between the conduction velocities of right sural nerves (p value0.007), right superficial peroneal nerve (p value 0.012) and bilateral tibial nerves (p values, 0.028 and 0.0075) in the groups with and without clinical neuropathy.

Table 3.1.2. Comparison of CMAP/SNAP amplitude between the two groups (with and without clinical neuropathy):

	Right			Left		
Sensory:	Group 1	Group 2	P value	Group1	Group 2	P value
Median	50.58(22.48)	36.37(22.17)		52.48(22.89)	41.19(17.75)	0.09
Radial	64.56(24.60)	45.39(20.39)		65.41(27.66)	40.88(17.75)	0.06
Sural	24.16(28.07)	8.65(8.56)	0.02	20.78(11.74)	11.11(7.84)	0.0025
Superficial peroneal	16.62(11.34)	8.92(11.14)	0.01	17.81(11.97)	10.32(10.05)	0.03
Motor:						
Median	7.99(2.35)	7.71(2.95)	0.72	7.29(2.61)	7.06(2.68)	0.75
Ulnar	8.75(3.04)	7.37(1.82)	0.07	8.39(2.59)	7.16(2.2)	0.09
Common peroneal	4.55(2.53)	3.69(2.69)	0.26	4.51(2.43)	4.57(4.81)	0.95
Tibial	6.27(1.95)	5.52(4.33)	0.42	6.08(2.15)	4.79(2.92)	0.08

The above table shows that there is a significant difference between the amplitudes of bilateral sural nerves (p values for right and left 0.02 and 0.0025 respectively) and bilateral superficial peroneal nerves (p values, 0.01 and 0.03) in the groups with and without clinical neuropathy.

3.2.Relation between Clinical Neuropathy based on MNSI(Michigan Neuropathy Screening Instrument) and Biothesiometer:

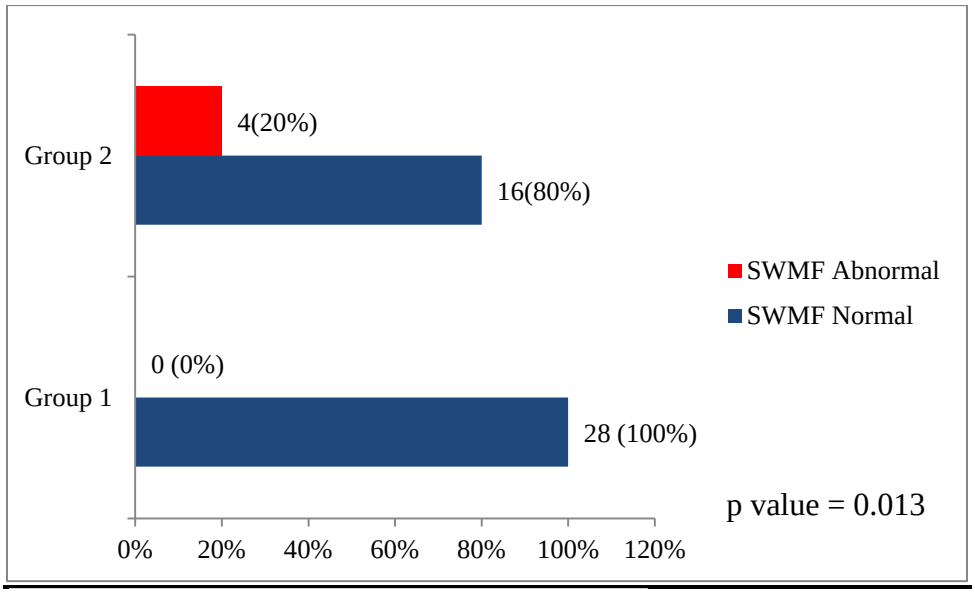
Table 3.2 Relation between Clinical Neuropathy based on MNSI(Michigan Neuropathy Screening Instrument) and Biothesiometer:

Biothesiometer	Group 1	Group 2
0 (<15 V) - Normal	18(64.29%)	3(15%)
1 (15-25V) - Mild	8(28.57%)	10(50%)
2 (25-40V) - Moderate	1(3.57%)	2(10%)
3 (>40) - Severe	1(3.57%)	5(25%)

P value = 0.001

The above table shows that in the group with no clinical evidence of neuropathy, 64.29% have no evidence of neuropathy by biothesiometry, and 85% of the patients in the group with clinical neuropathy also have abnormal biothesiometry. The correlation was significant with a p value of 0.001.

3.3. Relation between Clinical Neuropathy based on MNSI(Michigan Neuropathy Screening Instrument) and Semmes Weinstein Monofilament testing:

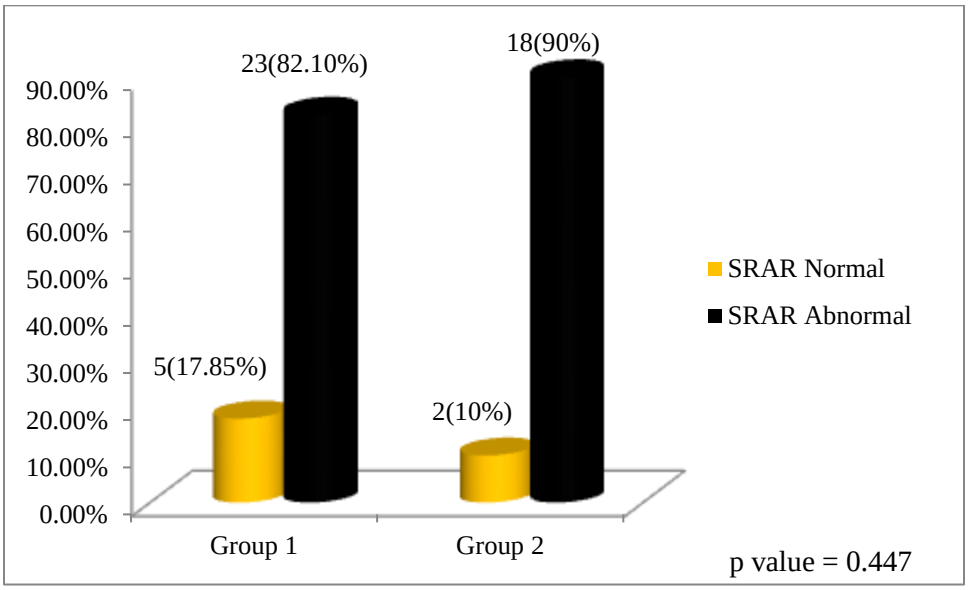


*SWMF - Semmes Weinstein

Fig 3.3 Bar graph showing Relation between MNSI and Semmes Weinstein Instrument

The above graph shows that all patients without clinical neuropathy and 80% of the patients with clinical evidence of neuropathy were able to perceive a 2 gram Semmes WeinsteinMonofilament at more than 6 out of 10 points. Four patients had neuropathy based on Semmes Weinstein monofilament and all four of them belonged to the group with clinical neuropathy based on Michigan Neuropathy Screening Instrument.

3.4.Relation between Sural Radial Amplitude Ratio(SRAR) and clinical neuropathy by Michigan Neuropathy Screening Instrument:

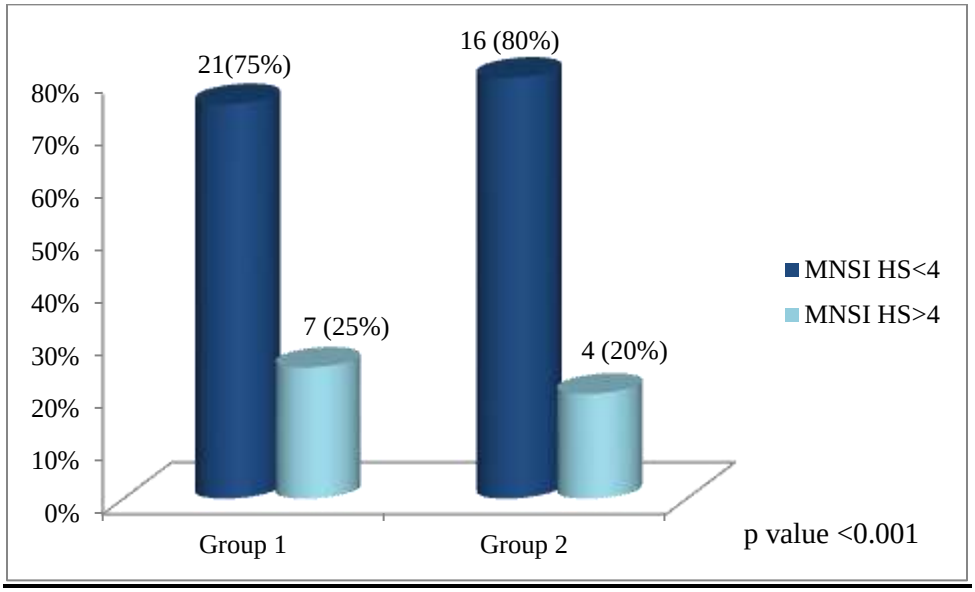


*SRAR - Sural Radial Amplitude ratio

Fig 3.4.Bar graph showing the Relation between Sural Radial Amplitude Ratio(SRAR) and clinical neuropathy by Michigan Neuropathy Screening Instrument

This shows that only 2 patients with clinical neuropathy have a normal SRAR, the remaining 90% have an abnormal SRAR. Also, 82.1% of patients with no clinical evidence of neuropathy have an abnormal SRAR.

3.5.Relation between symptoms based on Michigan Neuropathy Screening Instrument History Score(MNSI HS) and clinical neuropathy by Michigan Neuropathy Screening Instrument Examination Score(MNSI ES):



MNSI HS - Michigan Neuropathy Screening Instrument History Score

Fig 3.5.Relation between symptoms based on Michigan Neuropathy Screening Instrument History Score(MNSI HS) and clinical neuropathy by Michigan Neuropathy Screening Instrument Examination Score(MNSI ES):

The above bar graph shows that 75% of the patients in group 1 and 80% of the patients in group 2 have a normal history score of less than 4. This correlation is not statistically significant with a p value of 0.68.

3.6.Relation between Nerve conduction studies and biothesiometer:

Table 3.6.1. Relation between Nerve conduction studies and biothesiometer:

	NCS normal	NCS abnormal
Biothesiometer Normal	18 (52.94%)	3 (21.43%)
Mild neuropathy	14 (41.18%)	4 (28.57%)
Moderate neuropathy	0 (0%)	3 (21.43%)
Severe neuropathy	2 (5.88%)	4 (28.57%)

When we compare nerve conduction studies and biothesiometer,32 out of 34 (94.11%) patients with normal NCS have either no neuropathy or mild neuropathy according to biothesiometry. Of the 14 patients with abnormal NCS, 11(78.57%) could be picked up by biothesiometry.

The agreement between biothesiometer and NCS is 79.17% with a kappa of 44.95.

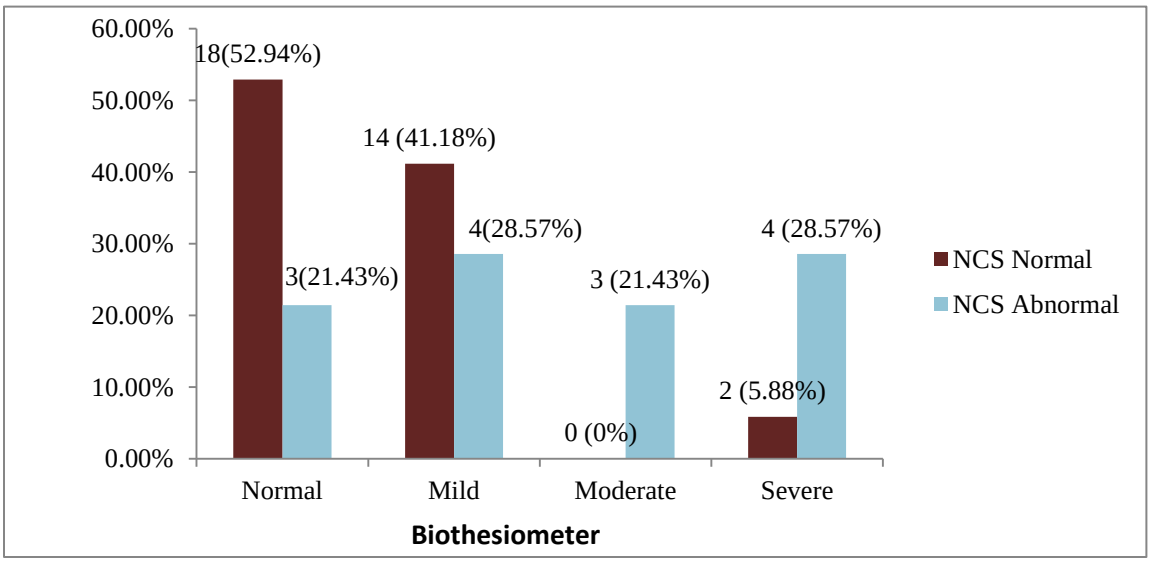


Fig.3.6.1 Relation between Nerve conduction studies and biothesiometry

3.6.2 Comparison of conduction velocity between two groups based on biothesiometer

	Right			Left		
	BT Normal	BT abnormal	P value	BT Normal	BT abnormal	P value
Sensory:						
Median	52.53(7.93)	45.4(16.33)	0.05	52.61(6.35)	31.33(12.04)	0.046
Radial	62.25(7.39)	60.72(13.43)	0.63	60.91(8)	59.59(13.98)	0.69
Sural	47.21(15.73)	13.4(21.69)	0.000	46.99(10.05)	28.53(24.97)	0.0007
Superficial peroneal	44.37(24.62)	9.44(20.14)	0.00015	45.03(21.19)	26.54(25.72)	0.023
Motor:						
Median	52.64(4.76)	44.01(14.25)	0.0025	52.54(6.09)	49.23(3.41)	0.11
Ulnar	57.40(6.78)	51.77(4.86)	0.018	57.15(6.78)	50.05(5.23)	0.0036
Common peroneal	44.41(4.61)	36.8(7.41)	0.0003	44.07(5.78)	36.52(6.52)	0.0013
Tibial	42.93(5.57)	37.06(5.18)	0.0043	43.57(4.37)	37.78(7.42)	0.011

BT - Biothesiometer

The above table shows that there is a significant difference between the conduction velocities of bilateral sural nerves (right and left p values, 0.00 and 0.0007 respectively),bilateral superficial peroneal nerves (p values, <0.001 and 0.023), bilateral common peroneal nerves (p values, <0.001 and 0.0013), bilateral tibial nerves (p values, 0.004 and 0.01) in the groups with and without neuropathy based on biothesiometer (25V as cut off).

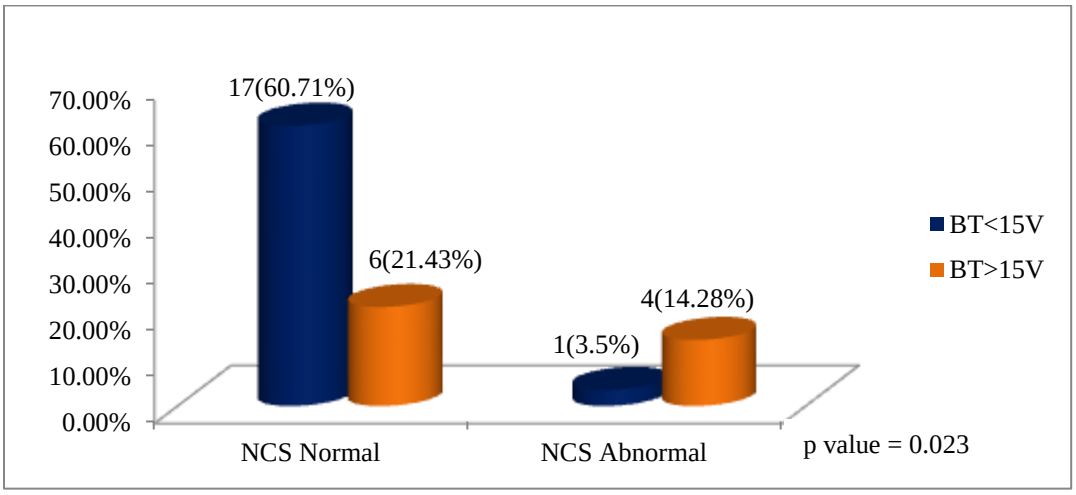
3.6.3 Comparison of amplitude between two groups based on biothesiometer:

	Right			Left		
Sensory:	BT Normal	BT abnormal	P value	BT Normal	BT abnormal	P value
Median	50.43(21.89)	22.73(13.02)	0.0004	52.61(6.35)	47.59(8.82)	0.005
Radial	62.60(22.92)	33.65(16.52)	0.0005	61.007(26.62)	33.09(11.88)	0.002
Sural	21.17(24.74)	4.5(8.35)	0.042	19.13(10.86)	7.69(8.02)	0.0032
Superficial peroneal	15.96(10.96)	3.72(9.92)	0.002	16.5(11.77)	7.8(8.96)	0.035
Motor:						
Median	8.16(2.74)	6.75(1.54)	0.13	7.41(2.71)	6.34(2.11)	0.24
Ulnar	8.34(2.79)	7.52(2.09)	0.39	8.1(2.52)	7.01(2.28)	0.22
Common peroneal	4.57(2.61)	2.74(2.14)	0.05	5.01(3.69)	2.74(2.41)	0.07
Tibial	6.60(3.03)	3.49(2.33)	0.004	6.07(2.43)	3.52(2.01)	0.003

BT - Biothesiometer

The above table shows that there is a significant difference between the amplitudes of bilateral sural nerves (0.04 and 0.003),bilateral superficial peroneal nerves (0.002 and 0.035), bilateral tibial nerves (0.004 and 0.003), bilateral median nerves (<0.001 and 0.005), bilateral radial nerves (<0.001 and 0.002) in the groups with and without neuropathy based on biothesiometer (25V as cut off).

3.6.4.Relation between Nerve conduction studies and biothesiometer in the group without clinical neuropathy:

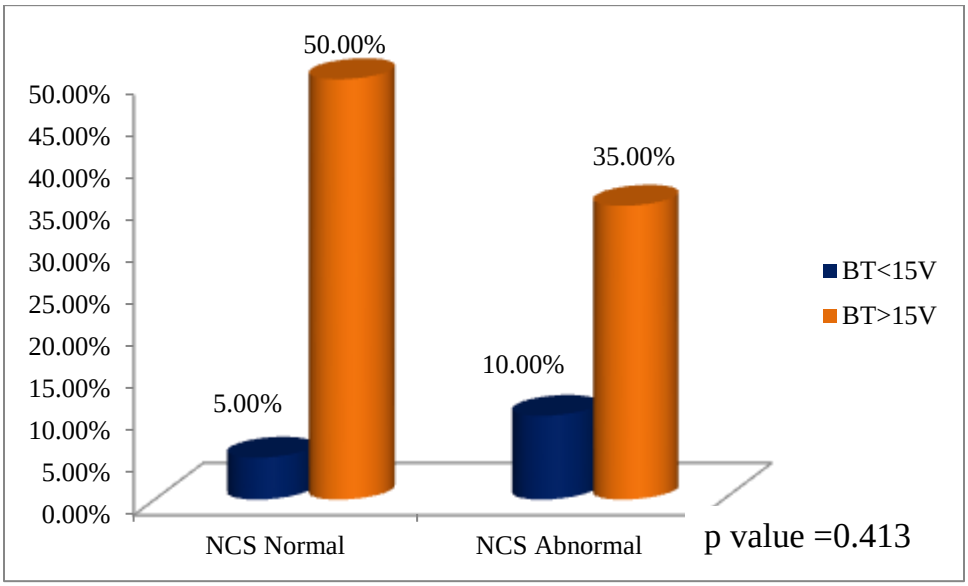


*NCS - Nerve conduction studies

Fig 3.6.4.Bar graph showing the relation between Nerve conduction studies and biothesiometer in the group without clinical evidence of neuropathy

The above table shows that in the group of 28 patients without clinical evidence of neuropathy 17 out of 28 patients(60.71%) have both normal NCS and biothesiometer recordings, while 4 out of 28 patients(14.28%) have abnormal NCS and biothesiometer recordings, that is they have clinically silent neuropathy according to biothesiometry well as NCS. The correlation was statistically significant(p= 0.023) However 6 patients (21.43%) have a normal NCS despite an abnormal biothesiometer value and one patient has an abnormal NCS despite normal biothesiometer value. Of the 6 patients with a normal NCS and abnormal biothesiometer value, 5 of them have mild neuropathy according to biothesiometer suggesting that mild neuropathy is better picked up by biothesiometer.

3.6.5.Relation between Nerve conduction studies and biothesiometer in the group with clinical evidence of neuropathy:



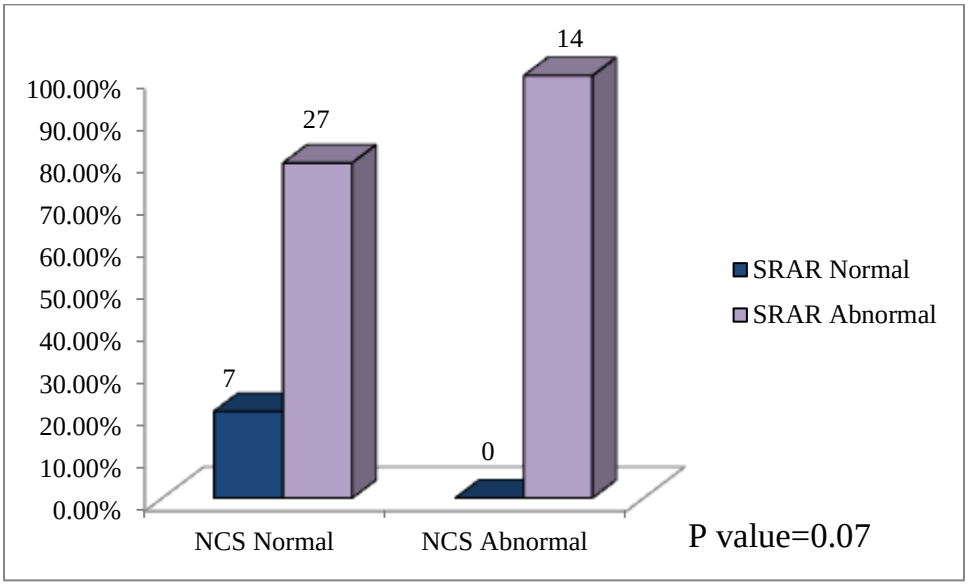
*NCS - Nerve conduction studies, BT Biothesiometer

Fig. 3.6.5.Bar graph showing relation between Nerve conduction studies and biothesiometer in the group with clinical evidence of neuropathy

This table shows that among the 20 patients with clinical evidence of neuropathy, 7 patients (35%) have both abnormal NCS and biothesiometer, while 10 patients, (50%)have an abnormal biothesiometer value despite a normal NCS. This shows that biothesiometer has picked up more cases of neuropathy in the clinically evident as well as clinically silent group.

4. USEFULNESS OF SRAR AND MINIMAL F WAVE LATENCY:

4.1.Comparison of Conventional Nerve conduction studies (NCS) and Sural Radial Amplitude Ratio(SRAR):



*NCS - Nerve conduction studies** SRAR - Sural Radial Amplitude Ratio

Fig 4.1. Comparison between Conventional nerve conduction studies and SRAR

This bar graph shows that every patient with an abnormal NCS also had an abnormal Sural Radial Amplitude ratio (100%). Among patients with a normal NCS also, 80% have an abnormal SRAR. Considering nerve conduction studies as the gold standard, the sensitivity of Sural Radial Amplitude ratio is 100% and the Specificity is 20.6%. It has a positive predictive value of 34.1% and a negative predictive value of 100%, which means that a person with a normal Sural Radial Amplitude ratio definitely doesn't have the disease, however if a person has an abnormal Sural Radial amplitude ratio, there is only 34.1% that he is truly diseased.

4.2. Comparison between NCS and F wave

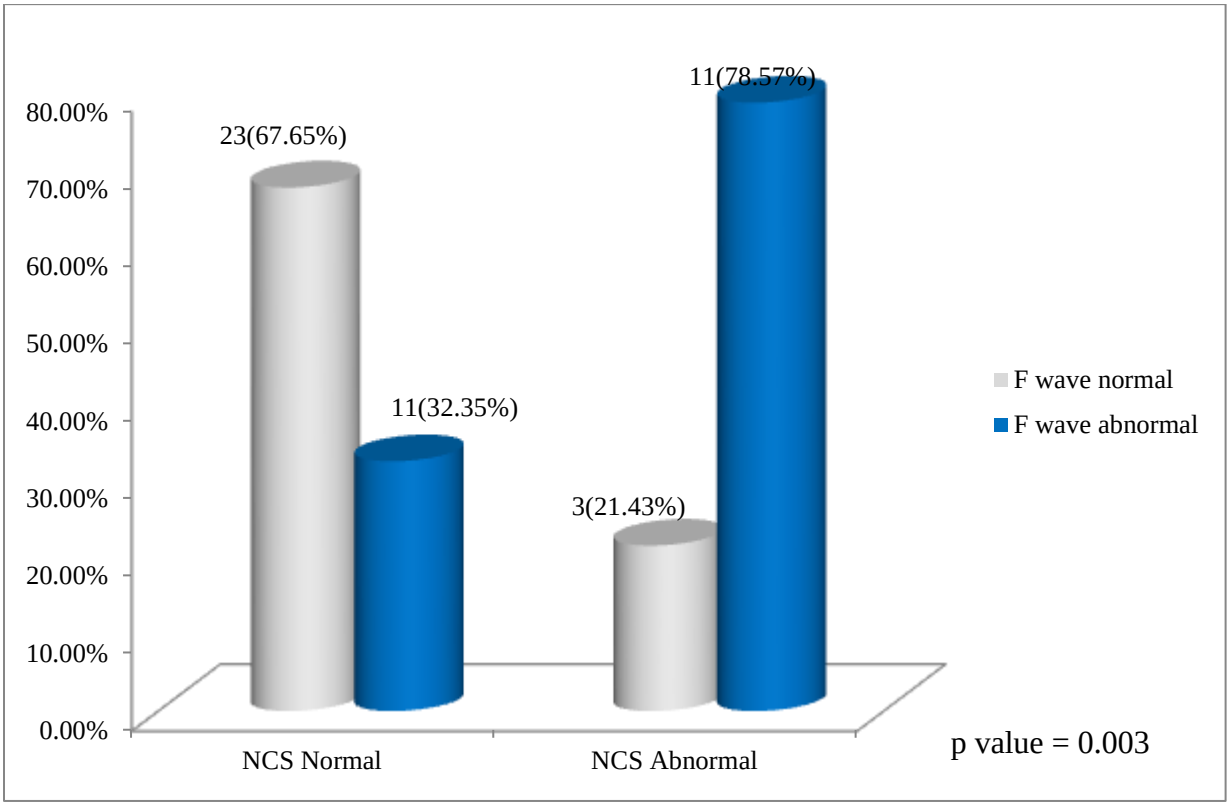


Fig.4.2.Comparison between NCS and F wave

The above graph shows that 67.65% of the patients with normal conventional nerve conduction studies also have normal F waves and 78.57% of the patients with abnormal NCS also have abnormal F waves. This is statistically significant with a P value of 0.003.

Table 4.3. Comparison of minimal F wave latency between two groups (with and without clinical neuropathy):

	Right			Left		
	Group 1	Group2	P value	Group 1	Group 2	P value
Median	26.84(1.86)	28.17(4.35)	0.16	27.11(2.24)	27.06(2.43)	0.95
Ulnar	27.19(2.14)	27.77(2.26)	0.37	27.48(2.22)	28.12(3.02)	0.40
Common Peroneal	46.69(6.86)	50.65(11.01)	0.13	48.34(6.88)	51.87(13.06)	0.18
Tibial	46.69(7.32)	51.16(8.85)	0.06	47.09(7.09)	52.90(11.83)	0.04

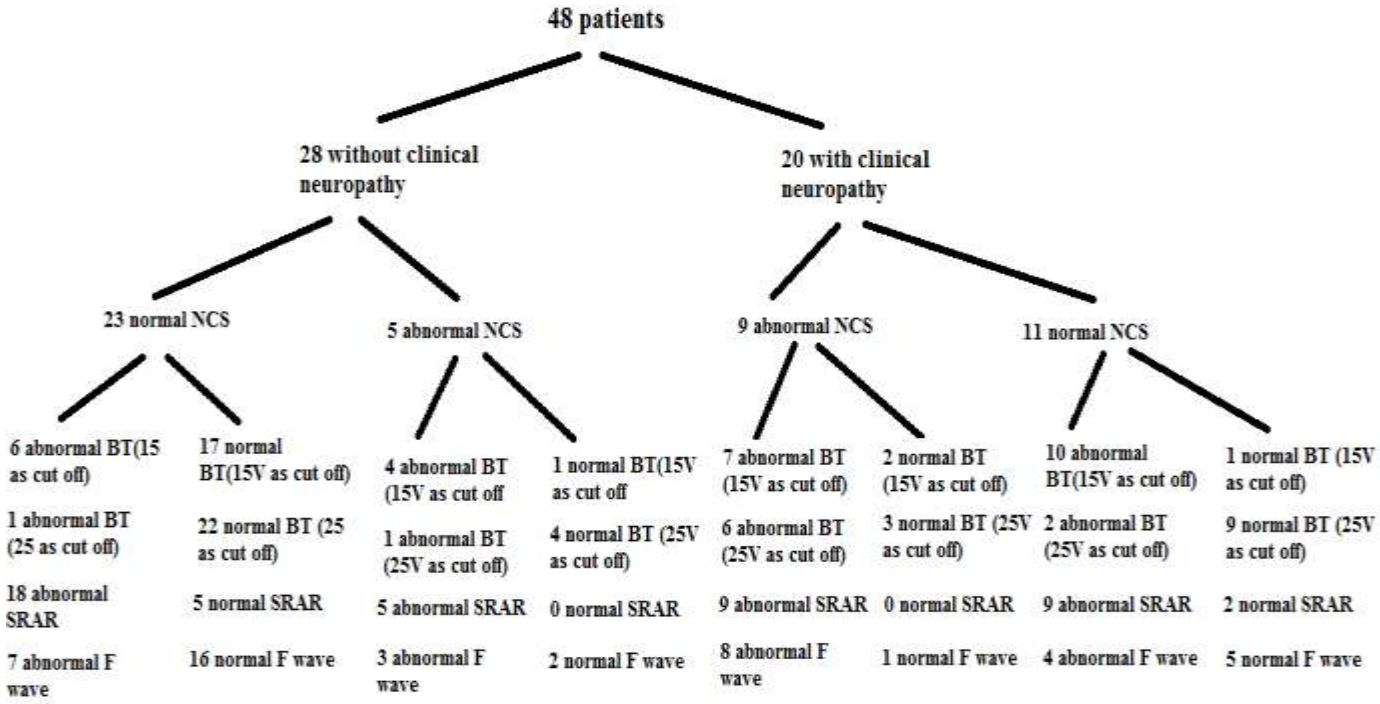
The above table shows that the minimal F wave latency is slightly prolonged in the group with clinical neuropathy than the group without clinical neuropathy, but significance was observed only for left tibial nerve (0.04).

Table 4.4. Comparison of minimal F wave latency between two groups based on biothesiometry:

	Right			Left		
	BT Normal	BT abnormal	P value	BT Normal	BT abnormal	P value
Median	26.64(1.49)	30.27(5.65)	0.0008	26.81(2.08)	28.15(2.83)	0.099
Ulnar	27.02(2.02)	28.99(2.18)	0.009	27.08(2.11)	30.28(2.71)	0.00021
Common Peroneal	45.96(7.46)	57.37(8.63)	0.0001	46.81(7.24)	61.21(10.96)	0.000
Tibial	46.67(6.80)	55.67(9.52)	0.001	46.65(7.01)	60.40(11.03)	0.000014

BT - Biothesiometer

The above table shows that the difference of mean minimal F wave latency between the two groups with and without neuropathy based on biothesiometry was significant for all nerves.



NCS - Nerve Conduction Studies; BT - Biothesiometer; SRAR - Sural Radial Amplitude Ratio

Flow chart showing the distribution of abnormalities in various screening tests

Of the total 48 patients, 28 of them didn't have clinical evidence of neuropathy and 20 of them had clinical evidence of neuropathy.

In the group with clinical evidence of neuropathy, 9 patients (45%) had an abnormal NCS, out of which all the 9 had an abnormal SRAR, 8 of them had an abnormal F wave and 7 of them had a biothesiometer value of more than 15V. Out of the 11 patients (55%) with a normal NCS, 10 of them had abnormal biothesiometry (cut off of 15V) and 9 of them had abnormal SRAR.

The group without clinical evidence of neuropathy is more important as it is the target group, where clinically silent neuropathy needs to be picked up. Of the 28 patients without clinical evidence of neuropathy, 5 of them have an abnormal NCS, 10 have abnormal biothesiometer recordings, 23 have abnormal SRAR values and 10 have abnormal F wave latencies. Of the patients with abnormal NCS, all 5 have an abnormal SRAR and 4 of them have a biothesiometer value more than 15V.

However, of these 28 patients, 23 of them have a normal NCS. Eighteen of them are picked up as abnormal by SRAR, 7 have an abnormal F wave and 6 of them have a biothesiometer value more than 15V.

5. SENSITIVITY AND SPECIFICITY OF THE VARIOUS SCREENIG TOOLS:

Table 5.1.Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) based on Nerve Conduction Studies (NCS) as the gold standard:

	MNSI	SWMF	BT(15V as cut off)	BT(25 V as cut off)	SRAR	F wave
Sensitivity	64.3%	14.3%	**78.6%**	50%	**100%**	**78.6%**
Specificity	67.6%	**95.1%**	52.9%	**91.2%**	20.6%	67.6%
Positive Predictive Value	45%	**50%**	40.7%	**70%**	34.1%	28.2%
Negative Predictive Value	82.1%	72.7%	**85.7%**	81.6%	**100%**	69.8%

MNSI - Michigan Neuropathy Screening Instrument; SWMF - Semmes Weinstein Monofilament; BT – Biothesiometer; SRAR - Sural Radial Amplitude Ratio

The above table shows that the most sensitive test is Sural Radial amplitude ratio, with 100% sensitivity and 100% negative predictive value. Biothesiometer with 15V as cut off has a sensitivity of 78.6% and negative predictive value of 85.7%. The highest specificity, 95.1% is for Semmes Weinstein monofilament, however the sensitivity is only 14.3%. Biothesiometer with a cut off of 25V has a specificity of 91.2% and a negative predictive value of 70%.

Table 5.2.Sensitivity and Specificity of combined parameters based on NCS as gold standard:

	SRAR + BT (15V as cut off)	SRAR + BT(15V as cut off) + MNSI	SRAR + BT(25V as cut off)
Sensitivity	78.6%	50%	50%
Specificity	61.8%	76.5%	97.1%

BT - Biothesiometer; SRAR - Sural Radial Amplitude Ratio; MNSI - Michigan Neuropathy Screening Instrument

	SRAR + F wave	SRAR + F wave + BT(15V)	SRAR + F wave + BT(15V as cut off) + MNSI
Sensitivity	78.6%	71.4%	57.1%
Specificity	82.4%	91.2%	91.2%

BT - Biothesiometer; SRAR - Sural Radial Amplitude Ratio;MNSI - Michigan Neuropathy Screening Instrument

By combining Sural Radial Amplitude ratio with biothesiometer(15V as cut off), there is a considerable increase in specificity to 61.8%, with only a slight decrease in sensitivity, to 78.6%. The highest specificity 97% is got by combining Biothesiometry of 25V with SRAR. The highest sensitivity for the combined tools is for SRAR with Biothesiometer with 15V as cut off (78.6%) and SRAR with F wave(78.6%).

6. NERVE CONDUCTION ABNORMALITIES IN INDIVIDUAL NEREVES:

Table 6. Nerve conduction abnormalities in upper and lower limb nerves:

PARAMETER AFFECTED	NUMBER OF PATIENTS(%)
UPPER LIMB NERVES	
Median sensory amplitude	2 (4.2%)
Median Sensory conduction velocity	12 (25%)
Radial sensory amplitude	0 (0%)
Radial sensory conduction velocity	0 (0%)
Median Motor amplitude	1 (2.1%)
Median motor conduction velocity	7 (14.58%)
Ulnar amplitude	3 (6.25%)
Ulnar conduction velocity	6 (12.5%)
LOWER LIMB NERVES	
Sural Sensory amplitude	13 (27%)
Sural sensory conduction velocity	10 (20.8%)
Superficial peroneal sensory amplitude	14 (29.1%)
Superficial peroneal sensory conduction velocity	16 (33.33%)
Tibial amplitude	8 (16.6%)
Tibial conduction velocity	6 (12.5%)
Common Peroneal amplitude	16 (33.33%)
Common peroneal conduction velocity	18 (37.5%)

The above table shows that lower limb nerves are more affected than the upper limb nerves, with the common peroneal nerve being the most commonly affected, followed by superficial peroneal nerve and sural nerve.

7. CORRELATION BETWEEN AMPLITUDES AND CONDUCTION VELOCITIES:

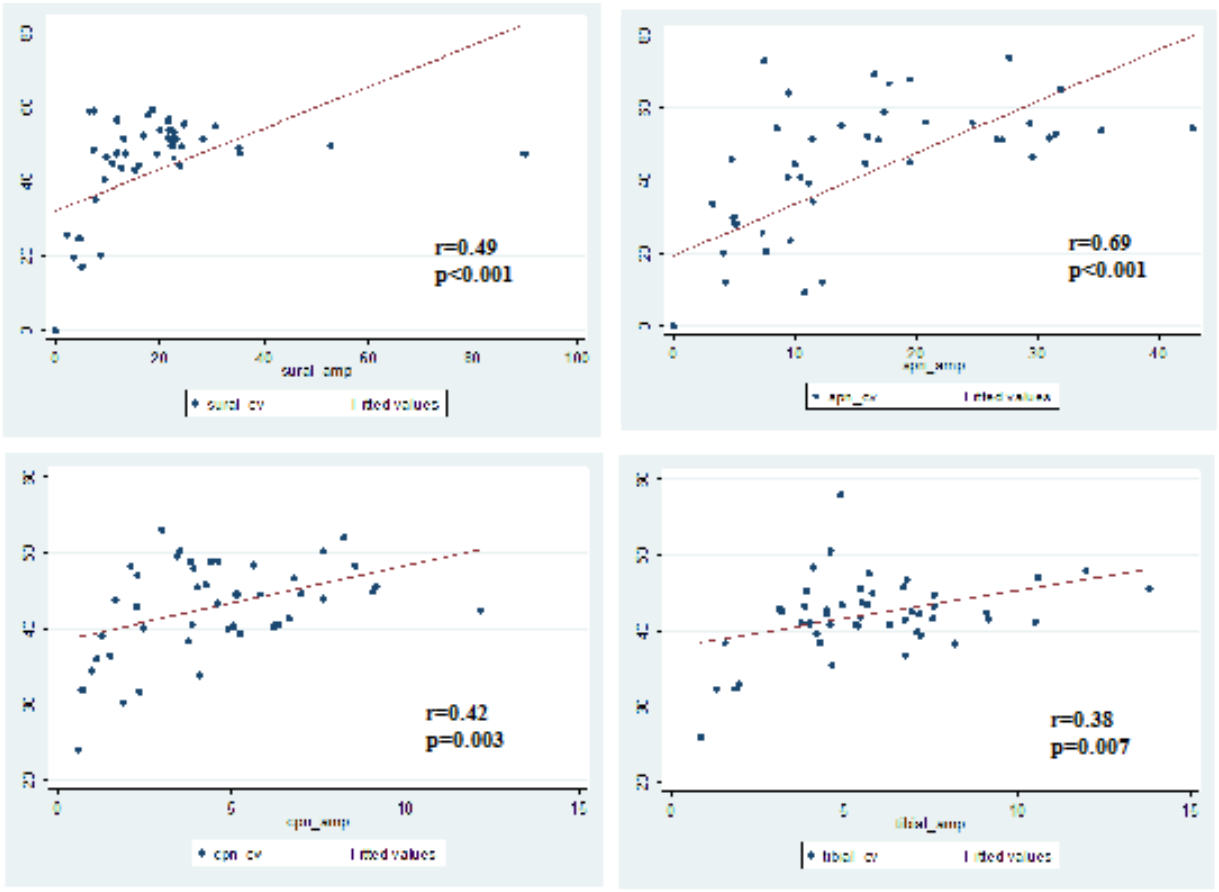


Fig 7.1:Correlation between amplitudes and conduction velocities for lower limb nerves

The above graph showed that there was a significant correlation between conduction velocities and amplitudes of all lower limb nerves with the highest for Superficial

peroneal nerve (correlation coefficient of 0.69 and p<0.001), followed by sural nerve (correlation coefficient of 0.49 and p<0.001).

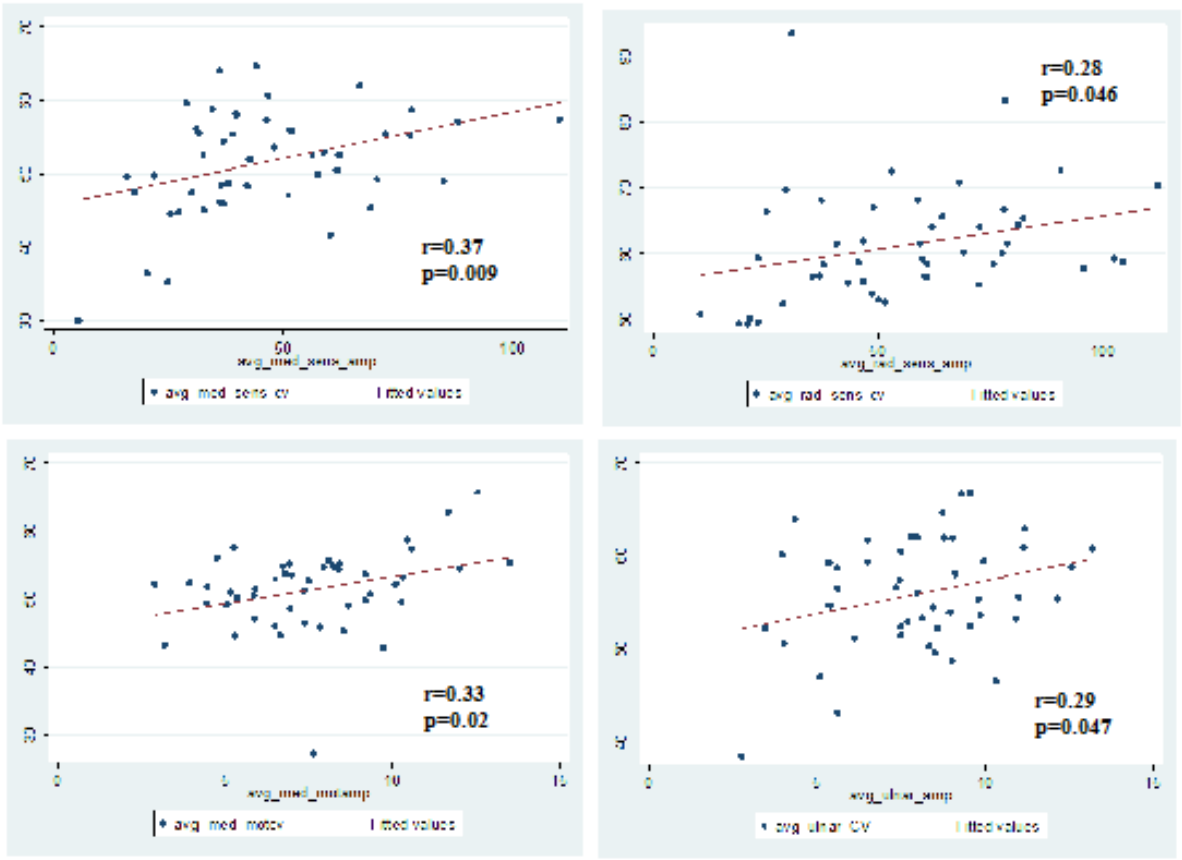


Fig 7.2:Correlation between amplitudes and conduction velocities for upper limb nerves

The above graph showed that there was a significant correlation between conduction velocities and amplitudes of all upper limb nerves with the highest for median sensory nerve (correlation coefficient of 0.37 and p value of 0.009).

8. ASSOCIATIONS WITH PERIPHERAL NEUROPATHY:

Table 8.1.Association of significant variables:

Variables	NCS Normal Mean(SD)	NCS Abnormal Mean(SD)	P value
Age	49.41(7.32)	55.93(7.56)	0.0079
BMI	24.75(2.89)	25.43(3.27)	0.48
Duration of Diabetes	5.27(4.09)	7.61(6.09)	0.31
HbA1C	7.72(1.83)	9.38(1.85)	0.0068
MNSI HS	2.12(1.55)	2.21(1.63)	0.799

BMI - Body mass Index, MNSI HS- Michigan Neuropathy Screening Instrument History score

The above table shows that age and HbA1C have a significant association with (P values of 0.0079 and 0.0068 respectively) the presence of neuropathy diagnosed on the basis of nerve conduction studies.

However, duration of diabetes and BMI do not significantly affect the presence of neuropathy based on nerve conduction studies.

Gender also did not show a significant association with the presence or absence of neuropathy. (P value = 0.48).

8.2.Multivariate Logistic Regression for significant variables:

Multivariate logistic regression was done for the two variables which had a significant correlation on univariate logistic regression; namely age and HbA1C.

Table 8.2.Multivariate Logistic Regression for significant variables:

Variable	Odds Ratio(95%CI)	P value
Age	1.16(1.04 o 1.29)	0.007
HbA1C	1.85(1.18 to 2.9)	0.008

The above table shows that for every 1 year increase in age, there is a 1.16 times more chance of developing neuropathy and with every one unit increase in HbA1C there will be 1.85 times more chance of developing neuropathy.

DISCUSSION:

This is an observational, cross sectional study comparing various screening tools for early detection of diabetic peripheral neuropathy. It is known that there is a wide variability in the prevalence of diabetic peripheral neuropathy due to the fact that different studies have used different screening tools. In the current study it has been observed that the prevalence of diabetic neuropathy has varied widely depending upon the method of diagnosis and the screening tool used.

Diabetic neuropathy was diagnosed in 29.16% patients with diabetes based on nerve conduction studies, which is the gold standard test. This was the same as two other Indian studies. Gill et al found a similar prevalence of 29.2% in Lucknow, however their definition of neuropathy was based on Neuropathy symptom score(NSS) and Neuropathy Disability Score(NDS). (28) Dutta et al in his study among patients with recently diagnosed diabetes mellitus from Manipur also showed a similar prevalence of 29%. Neuropathy was diagnosed based on NSS, NDS and abnormal common peroneal nerve conduction studies.(6) Bagchi et al in his study among 50 normal people and 50 patients with diabetes reported a prevalence of neuropathy of 44% in diabetics based on nerve conduction studies. The NCS was interpreted according to the American Academy of Neurology protocol, which is the same criteria used in our study.(65)

A recent study showed that the prevalence of diabetic neuropathy was 97% according to nerve conduction studies. The reason for this wide difference could be the fact that they had taken nerve conduction studies as abnormal if any one parameter of any nerve tested

were abnormal, however as per our criteria at least two parameters had to be affected and one of those should include the sural nerve. (66)

Of the total sample size of 48 patients, 58.33% had clinical neuropathy based on Michigan Neuropathy Screening Instrument with 2 as cut off in the examination score. Turkan mete et al reported that 32.07% of the patients had clinical neuropathy based on MNSI.(67) This was slightly lower than our study probably because the cut off score they used was 2.5 and our cut off was 2, hence the sensitivity was slightly lower.

A study aimed at validation of Michigan Neuropathy Screening Instrument showed that as the cut off for defining clinical neuropathy increased, there is a decrease in sensitivity and an increase in specificity(cut off of 2 and 2.5 have a sensitivity of 65% & 50% and a specificity of 83% & 91% respectively.(20) Al Geffari et al showed that 45% of the patients had neuropathy according to MNSI. (30)

In the current study, 56.25% of the patients had an abnormal biothesiometer value of more than 15 Voltsand 18.75% of the patients had a biothesiometer value of more than 25 volts. This is similar to Young et al's study on 469 patients in which 55.44% of the patients had a biothesiometer value of more than 15 V.(34) A study done in Post Graduate Institute of Medical Education and Research, in Chandigarh on validation of bedside methods in evaluation of diabetic peripheral neuropathy, had taken biothesiometer as gold standard and compared other tools like Diabetic neuropathy symptom score and examination score with biothesiometer. They had found that 34.9% of the patients had a biothesiometer value of more than 25 Volts. The reason for this difference could be because of the difference in the method of application. They had

applied the biothesiometer over the plantar aspect of the great toe, while we had applied it over the dorsum of the great toe. They had also included patients with ulcers, however we had excluded them. Another reason could be that their sample size was 1044 patients, much higher than our sample size of 48 patients.(32)

In our study, among patients with clinical neuropathy, 45% had abnormal NCS and among patients without clinical neuropathy, 18% had abnormal NCS. Among patients with abnormal nerve conduction studies, 64% of patients had clinical neuropathy. According to NCS as gold standard, MNSI has a sensitivity of 64.3%, specificity of 67.6%, positive predictive value of 45% and Negative predictive value of 82.1%. Another study done by Turkaan mete et al had similar results where, among patients with clinical neuropathy, 58.8% had abnormal NCS(68).

Many studies use NCS as the gold standard and have validated all tools based on NCS.(4,12–14)However there have been suggestions that NCS alone is not enough for the diagnosis of Diabetic peripheral neuropathy, it should be supplemented by clinical evidence. (48)

Even, MNSI, a clinical tool alone is not sufficient to diagnose Diabetic peripheral neuropathy. However, Turkan Mete et al showed that NCS had a sensitivity of 55% and a specificity of 58% when compared to MNSI and the positive and negative predictive values were 38% and 73% respectively(67). In our study NCS was found to have a slightly lower sensitivity of 45% and a better specificity of 82.1%. The reason for this could again be, as mentioned above, the fact that they had taken 2.5 as cut off for MNSI, so their percentage of patients with clinical neuropathy was lower and hence the

percentage of patients with abnormal NCS among them are also lesser than ours. The reason for the low sensitivity of NCS as compared to MNSI could be that small fiber neuropathy is generally not diagnosed by nerve conduction studies and needs other tools like thermal testing. (29)

Fateh et al compared UK Screening Test and MNSI with electrodiagnosis and found that according to MNSI, 69% of the patients had clinical neuropathy. The sensitivity for MNSI was 75.21% and specificity was 33.3%.(66) Herman et al showed that MNSI had a sensitivity of 61% and a specificity of 79% with a cut off of >2.5 of the examination score; with respect to clinical examination by a neurologist and NCV with >/= 2 parameters affected of all the upper and lower limb conventional nerves tested. The sensitivity is almost the same, however our study had a slightly lower specificity, the reason for this could be the cut off taken as >2.5, while our cut off was 2.(71) As previously mentioned, studies have shown that higher the cut off, higher is the specificity. (20)

A study by Muntean et al, published in 2016 February, studied the efficiency of MNSI and NCV in the early diagnosis of peripheral neuropathy. The prevalence according to MNSI was 50.98% which was similar to our finding of 56.25%.(72) In this study they have done Nerve Conduction studies for all patients, however they have not used a single criteria which defines whether the NCS is normal or not. They have compared the NCS of patients and controls and found a significant difference in certain parameters. For MNSI questionnaire,the prevalence of diabetic peripheral neuropathy was 3.92% when keeping the old threshold(>/=7), and 23.52% when using the modified

threshold(>/=4)(72). In our study, the maximum MNSI history score was 5. Hence there were no patients with score of 7 or more. The prevalence increased from 0% to 22.91% when the cut off was changed to >/=4. This finding is similar to the above mentioned study.

Gefarri et al showed that the prevalence of diabetic peripheral neuropathy based on MNSI was 45%. Out of this 81.7% had symptoms, however only 7.4% met the questionnaire criteria for neuropathy based on MNSI (history score of >/=7).(30)

In the current study, of the total 28 patients without clinical evidence of neuropathy, biothesiometer has identified 10 patients (35.71%) to have evidence of large fibre neuropathy, and in the group with clinical evidence of neuropathy 17 patients(85%) were identified to have neuropathy based on biothesiometry; by taking the cut off for definition of abnormal as 15 V. In the clinically silent group, 23 patients out of 28 have a normal NCS. Of these 23, 6 patients are picked up as abnormal by biothesiometer. Of the 5 patients who have an abnormal NCS, 4 of them are picked up by biothesiometer. Of the 20 patients in the group with clinical neuropathy, 9 patients have an abnormal NCS, and of these 7 are picked up as abnormal by biothesiometry. Ten out of the eleven patients with a normal NCS in the clinical neuropathy group, have an abnormal biothesiometry. What is evident here is that there are more number of patients picked up by biothesiometry than by NCS; and this number is higher in the group with clinical neuropathy (90.9%) than in the group without clinical neuropathy(26%).This shows that

biothesiometry correlates more closely with clinical neuropathy than Nerve conduction studies.

It is also observed here that 80-85% of the patients with a normal NCS and an abnormal biothesiometer value belong to the mild neuropathy category by biothesiometry(15-25V). This also shows that more number of patients with mild neuropathy diagnosed by biothesiometry are being missed by nerve conduction studies. It could also mean that these are false positive cases and actually don't have neuropathy because nerve conduction studies are normal. Here arises the question as to which is the gold standard. There are no studies so far which have directly compared Nerve conduction studies and biothesiometer. Some consider biothesiometer as the gold standard(32,73) while others consider nerve conduction studies as the gold standard.(33,66,69,70) According to other authors, even nerve conduction studies alone are not sufficient; there must be clinical evidence and electrophysiological evidence to diagnose diabetic neuropathy(48). Ideally patients have to be followed up over years to see if they develop ulcers and the development of ulcer has to be taken as the gold standard. However, since ours is a cross sectional design, we have taken nerve conduction studies alone as gold standard, because it is purely an objective test and keeping the general consensus of majority of the studies. We have used the AAN criteria to define a case of neuropathy based on NCS. We have compared every other screening tool with NCS to determine their sensitivity and specificity.

After diagnosis of any condition, the next step would be management and prevention of complications. After diagnosis of diabetic neuropathy, management mainly comprises

prevention of complications like ulceration, gangrene and amputation. Medications need to be given only in the clinically symptomatic group. Strategies to prevent foot ulceration include foot care practices. Since the intervention after diagnosis is mainly knowledge and practice of foot care, the ultimate aim would be to pick up maximum number of cases, rather than strictly identify who is diseased and who is not. In fact, every patient with Diabetes mellitus, irrespective of whether he has neuropathy or not has to be taught foot care practices.

Biothesiometry with a cut off of 15V has a sensitivity of 78.6% and specificity of 52.9%. However if we increase the cut off to 25V the sensitivity decreases to 50% and the specificity increases to 91.2%.

A study on evaluation of clinical tools in diabetic peripheral neuropathy by Pouharmidi et al showed that the sensitivity and specificity of biothesiometry with a cut off of 24.5V was 82% and 70% respectively. The reason for this increased sensitivity could be the fact that they had a more strict criteria to define cases with peripheral neuropathy; that is clinical neuropathy by NDS (Neuropathy Disability score) and electrophysiological evidence of neuropathy. This study had also taken into consideration another aspect, small fiber neuropathy and had defined cases based on clinical evidence and thermal perception threshold. For small fiber neuropathy, the sensitivity and specificity of biothesiometer was 67% and 46% respectively despite taking the cut off as 20V(29). The reason for this is the vibration sense is a measure of large fiber function. This aspect was not looked at in our study.

Armstong et al showed that biothesiometry with a cut off of 15V had a sensitivity and specificity of 90% and 65% respectively. When the cut off value was increased to 25V the specificity increased to 85% whereas the sensitivity decreased slightly (85%)(31). These values are almost similar to our study, however, the slightly higher sensitivity and specificity in this study could be again due to the stricter criteria for defining cases, that is any patients with ulcers or history of ulceration. A study done by Mythili et al had shown that biothesiometry had a sensitivity and specificity of 86% and 76% respectively when NCS was taken as the gold standard(74).

Of the total sample size of 48 patients, only 4 (8.3%) of them had an abnormality in Semmes Weinstein monofilament testing. All the four patients belonged to the group with clinical neuropathy. The sensitivity and specificity of a 2 gram monofilament are 14.3% and 95.1% respectively; based on nerve conduction studies. The positive and negative predictive values are 50% and 72.7% respectively.

Pourhamidi et al, showed that the sensitivity and specificity of 10 gram monofilament was 6% and 97%. This was similar to our study. However they had not used 2 gram or 4 gram monofilaments. The gold standard used in this study was nerve conduction studies and clinical signs together(29).

A study done by Gill et al in PGI, UP, showed that that only 6.1% of the patients had an abnormality in 10 gram monofilament. However, in the same population Biothesiometer picked up 43% with 9 V as cut off. This finding was also similar to our study.

Jayaprakash et al showed that the sensitivity and specificity of 10 gram monofilament were 63% and 93% respectively with Vibration proprioception threshold according to

biothesiometry as gold standard while Armstrong et al with the gold standard as presence or absence of ulcer showed that the sensitivity and specificity for 10 gram monofilament with four points of testing were 90% and 85% respectively. Gefarri et al showed that the sensitivity and specificity were 69.7% and 87.9% respectively with MNSI as gold standard. Arshad and Alvi et al showed a sensitivity of 41.8% and specificity of 92.91% with VPT as gold standard.

There is wide variability among the sensitivity and specificity of 10 gram monofilament in different studies. The reason for this could be the fact that there is no standardization for the use of monofilament, that is, number of sites to be tested, the location of the sites, plantar or distal, the gold standard test used to compare and derive the sensitivity and specificity is variable among all these studies, hence it is very difficult to make a comparison. Other drawbacks could include lack of blinding, the test itself is a subjective one, the sole thickness varies from one ethnic population to another. Other factors influencing this are environmental factors such as effect of humidity, temperature, filament ageing and filament durability.

It is observed from the above quoted articles, that when the main outcome measure is the presence or absence of ulcer and this is taken as the gold standard, then the sensitivity and specificity is much higher. This implies that in advanced cases it is a good tool, however for newly diagnosed patients, or those with mild neuropathy it is not a very sensitive tool.

Studies where 10 gram monofilament has a very low sensitivity have suggested the use of a lesser calibre monofilament for earlier diagnosis. Studies have recommended that the

use of 20 different monofilaments is a more sensitive technique in picking up neuropathy earlier(75). However our study has shown that even 2 gram monofilament has a very low sensitivity and specificity.

In our study, we tried to analyze one more derived ratio from the nerve conduction studies, the sural radial amplitude ratio. Out of the total 48 patients, 85.42% of the patients had an abnormal sural radial amplitude ratio. Of the total patients with SRAR abnormality, 43% had clinical neuropathy and 56.09% didn't have clinical evidence of neuropathy. It was also observed that every patient with an abnormal NCS, also had an abnormal SRAR. Among those with a normal NCS also, 80% of the patients had an abnormal SRAR. Hence the sensitivity was 100% and the specificity was 91.2%; the positive and negative predictive values were 50% and 72.7% respectively.

Another parameter in nerve conduction studies useful in subclinical diagnosis of peripheral neuropathy is F wave. There was a good correlation between the conventional NCS and F waves with a p value of 0.003. Among the 14 patients with abnormal nerve conduction studies, 11 of them (78.57%) also had an abnormal F wave. Among the 34 patients with a normal NCS, 11 of them (32.25%) had an abnormal F wave.

We also found that by combining various screening tools there was a better specificity with only a slight decrease in sensitivity. The study with the highest sensitivity was Sural radial amplitude ratio(100%). Biothesiometer with a cut off of 25V had a high specificity of 91.2%, hence any patient with a biothesiometer value of more than 25V could be said to have neuropathy and does not need further investigation. However, on combining this with SRAR the specificity increases to 97%.

If a patient has a biothesiometry value of 15 to 25V, performing an additional SRAR would help. If SRAR is negative, we can be sure that he is not diseased. If SRAR is positive, there is an increase in specificity to 61.8%. Adding F wave to this combination would further increase the specificity to 91.2%.

If a patient has a normal biothesiometry value of less than 15V, then SRAR can be performed. If SRAR is normal then we can be sure that there is no neuropathy. However, if SRAR is positive, then the specificity is only 20%, hence an additional F wave is needed. If F wave is also positive then the specificity is 82.4%. If F wave is negative, then we need to perform the full NCS to be sure whether he has neuropathy or not.

Although Michigan Neuropathy Screening Instrument has an individual sensitivity and specificity of 64.3% and 67.6%, combining it with other screening tools does not increase the specificity much, however decreases the sensitivity.

Pourhamidi et al showed that combining with skin biopsy with biothesiometry, with either of the two positive as diseased led to identification of more cases. However adding tuning fork or Semmes Weinstein monofilament did not add to the sensitivity. (29)

Perkins et al showed that by combining the neuropathy score (4 query verbal symptom assessment), Semmes Weinstein monofilament and biothesiometry, there was an increase in the specificity to 89.4% and a decrease in the specificity to 86.7%. (33)

In our study the total prevalence of diabetic neuropathy based on nerve conduction studies was 29.1%, considering the AAN criteria where any two attributes of any two nerves, one being the sural nerve, had to be affected to define the patient to have Diabetic peripheral neuropathy. However, if we consider the individual parameters, the most

commonly affected parameters were Common peroneal nerve conduction velocity and amplitude (37.5% and 33.33%), followed by superficial peroneal nerve conduction velocity and amplitude (33.33% and 29.1% respectively). This is followed by sural nerve abnormalities in amplitude and conduction velocities(27% and 20.8%). Tibial nerve conduction velocity was abnormal in 12.5% of the patients and amplitude in 16.6% of the patients. A retrospective study done in 63799 electrophysiological counters showed that common peroneal nerve amplitudes were abnormal in 32.5% of the patients which was similar to our study. However they found 62.7% of the patients with a sural nerve abnormality.(76)

The upper limb motor and sensory nerves were much less affected when compared to lower limb nerves. This could be explained by the length dependent peripheral neuropathy in diabetes mellitus. The sensory sural and superficial peroneal nerve parameters were more affected than the radial or median nerve parameters. This finding was similar to a study done by Aruna et al in Telangana, India, published recently, in June 2016.(77) This study was done in 30 diabetic and 30 non diabetic patients and they also showed that tibial nerve was least affected among lower limb nerves, also similar to our study. This study has also shown that sensory nerves of lower limbs are more affected than the motor nerves. This is different from our finding where the most common abnormality was in the common peroneal motor conduction velocity and amplitude, slightly higher than the sural and superficial peroneal parameters. The fact to be noted here is that even though the number of patients with common peroneal nerve abnormalities is higher, when we look at the severity of the nerve involvement, there are

no patients with an absent common peroneal CMAPs, they only have a decreased amplitude or conduction velocity or both. However, even though only 29.16% of patients have abnormal sural NCS, 20% have absent SNAPs. Abnormal superficial peroneal SNAPs are found in 37.5% of the patients, and 33.33% of them have absent SNAPs. This indicates that the severity of sensory involvement is higher than that of motor involvement. Another study done by Bagchi et al has shown that there is a significant difference in both motor as well as sensory nerve parameters, even though it is commonly thought that motor nerves are rarely involved. (65)Even among the sensory nerves, it is noticed that superficial peroneal is more affected that sural nerve. The AAN criteria(42) and other studies say that sural is more affected than superficial peroneal(76). Lo et al has shown similar results as ours where superficial peroneal nerve is more affected than the sural nerve. They have shown that superficial peroneal nerve was affected in 89% of their patients, while sural nerve was affected only in 75% of the patients(78). The reason for this high percentage of patients being affected is that they have taken patients who have already been diagnosed with peripheral neuropathy. All their patients had parasthesia and distal motor and sensory signs and symptoms.

In our study, the common peroneal motor nerve conduction studies were more affected than the tibial NCS, keeping in line with centripetal progression of peripheral neuropathy. In our study, the amplitude and conduction velocity have a significant correlation with each other for almost all the nerves (p value of <0.001 for sural and superficial peroneal nerves, 0.003 for common peroneal nerve, 0.007 for tibial nerve). This is similar to another study done by Wilson et al.(79) Amplitude denotes the axonal continuity,

whereas conduction velocity denotes the degree of myelination. This shows that axonal loss is superimposed by demyelination in diabetic peripheral neuropathy. There could be another explanation to this, metabolic abnormalities in diabetes alter the sodium, potassium and calcium channels, which would affect the propagation of action potentials across the axon.(79)

Multiple logistic regression analysis was done for the parameters which were significant on univariate analysis and showed an increase in risk of diabetic peripheral neuropathy with increasing age(OR 1.16, 95% CI 1.04 to 1.29, p=0.007) and severity of diabetes, as measured by HbA1C (OR 1.85, 95%CI 1.18 to 2.9, p=0.008). It has been shown that even in the normal population, age has a significant influence on nerve conduction parameters.(27–29) It has been explained that this could be due to changes in nerve fibre membrane, decrease in the number of fibres and a decrease in the nerve fibre diameter, as age increases.(45)We had observed that there was increase in the occurrence of diabetic neuropathy as the age increased. Even though there is a general change in the nerve conduction parameters with increasing age, this difference is more pronounced in those with diabetes. Another study done by Gill et al also showed that there was a significant correlation with increasing age (OR 1.7, 95% CI: 1.2-2.5, P = 0.002)(28). Other studies also showed significance of age which is similar to our study.(6,7,34,44,65,77,80) Older people have to be routinely screened as it is very important to identify neuropathy early. They are more prone to ulceration and complications keeping in view the associated visual and vascular problems.

In our study, we have identified that the severity of diabetes based on HbA1C also had a

significant correlation with the occurrence of neuropathy. This was similar to some studies(7), however some other studies have said that this was not a significant correlation. (28)

Our study showed that the duration of diabetes doesn't significantly affect the presence or absence of neuropathy based on NCS. This was similar to a study done by Aruna et al. (77) However, some studies have shown that the duration of diabetes has a significant association with the occurrence of diabetic neuropathy. (6,7,80,81) This could be because duration of diabetes is not a reliable parameter as we don't know the actual duration of diabetes prior to the diagnosis. It would be more relevant to consider the duration of symptoms prior to the diagnosis of Diabetes mellitus. Some studies have shown that the duration of symptoms prior to diagnosis of diabetes caused an increased risk for diabetic neuropathy.(28) Another study, done by Novella et al showed that in patients with idiopathic sensory neuropathy there was a significant percentage with undiagnosed diabetes mellitus and impaired glucose tolerance.(82)However, in our study, we have not taken into account the duration of symptoms prior to the diagnosis of diabetes, and this would be a potential limitation. Other parameters like gender and BMI didn't correlate with the occurrence of neuropathy.

LIMITATIONS:

1. This was a pilot study and the sample size was small. More number of patients are needed to get a more accurate sensitivity and specificity of the various screening tools.

2. NCS is considered as the gold standard for the diagnosis of neuropathy. However some studies have quoted that NCS alone is not sufficient to diagnose peripheral neuropathy. The ideal gold standard would be development of ulcers. Hence it would have been good to follow up the patients with both NCS normal and abnormal findings and see which group develops more ulcers. By this the sensitivity and specificity of the various screening tools could be assessed. However, ours is a cross sectional observational study and we have not followed up patients.

3. Alcoholic patients were not excluded from the study and it could have been a confounding factor.

4. Duration of symptoms prior to the diagnosis of diabetes could have been looked into as it would be more relevant than the time since diagnosis.

5. Normal NCS is affected significantly by age and height. This was not taken into consideration. It would be more appropriate to take age and height corrected values of NCS while interpreting abnormal data.

CONCLUSIONS:

The following conclusions were drawn from the study:

1. Twenty nine percent of the patients with diabetes mellitus, without foot ulcers, were diagnosed to have peripheral neuropathy based on nerve conduction studies, 56.25% based on biothesiometer with 15V as cut off, 41.66% based on MNSI and 8.3% based on Semmes Weinstein monofilament testing.

2. There was a significant relation of MNSI with Nerve conduction studies (p=0.041), Semmes Weinstein monofilament testing (p=0.013) and biothesiometry (p=0.001). The agreement between MNSI and NCS was 66.67%

3. Of the patients with a normal nerve conduction study, 94.11% were found to have either no neuropathy or mild neuropathy according to biothesiometry. Of the 14 patients with abnormal NCS, 11(78.57%) could be picked up by biothesiometry. The relation between the results of NCS and biothesiometry was statistically significant in the group without clinical neuropathy (p value 0.023). The agreement between biothesiometer and NCS is 79.17%.

4. There was a statistically significant relation between neuropathy diagnosed by SRAR and by conventional NCS (0.07). Significant results were also noticed in the relation between neuropathy picked up by minimal F wave latency and conventional NCS (0.003). There was also a significant relation between the mean minimal F wave latencies of all nerves and biothesiometry. This shows that SRAR and minimal F wave latency are useful tools in diagnosing diabetic peripheral neuropathy.

5. The sensitivity and specificity of biothesiometer with a cut off of 25V was 50% and 91.2% respectively and when the cut off is reduced to 15V, the sensitivity increases to 78%, while specificity decreases to 52.9% with reference to NCS. The sensitivity and specificity of MNSI was 64.3% and 67.6% respectively. SRAR had the highest sensitivity of 100%, but a low specificity of 20% based on NCS as gold standard.

It may be concluded that if a patient has a biothesiometry value of more than 25V, no further testing is required as the specificity is 91.2%. If the biothesiometry value is 15 to 25V, performing an additional SRAR would help. If SRAR is negative, we can be sure that he is not diseased. If SRAR is positive, there is an increase in specificity to 61.8%. Adding F wave to this combination would further increase the specificity to 91.2%.

If a patient has a normal biothesiometry value of less than 15V, then SRAR can be performed. If SRAR is negative, we can be sure that he is not diseased. If SRAR is positive, then an additional F wave is needed. If F wave is also positive then the specificity is 82.4%. If F wave is negative, then we need to perform the full NCS to be sure whether he has neuropathy or not.

SRAR and biothesiometry are quick to perform. F wave study requires relatively longer time and expertise, however is less time consuming than the entire NCS.

Though NCS is considered the gold standard for diagnosis of diabetic peripheral neuropathy, it is cumbersome, time consuming, needs expertise and is painful to the patient although non invasive. Hence this study has shown that by combining biothesiometry, SRAR and F wave studies, the need for conventional NCS can be limited to selected cases.

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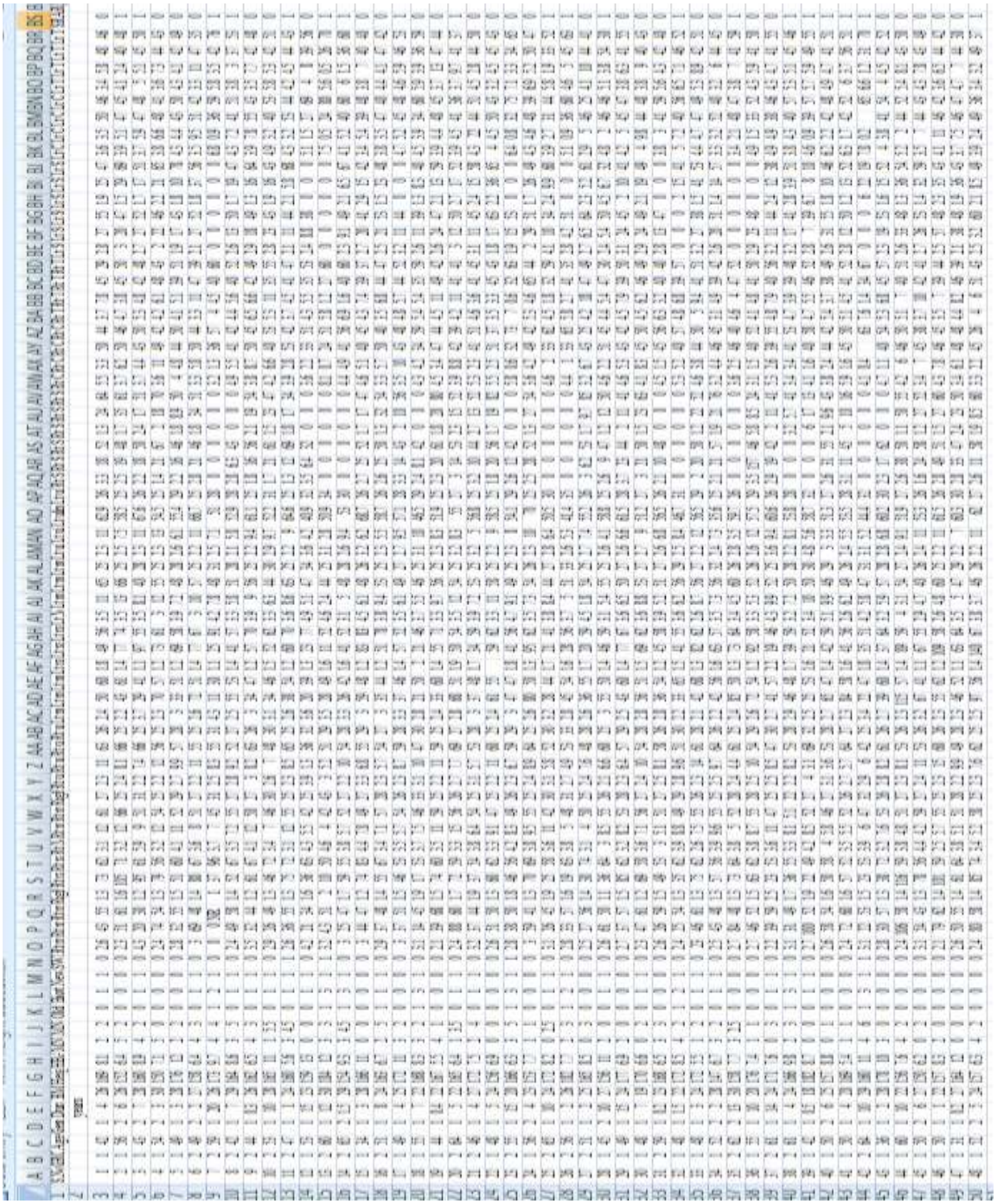
ANNEXURES:

ANNEXURE 1: THESIS DATA(48 patients):



ANNEXURE 2: IRB APPROVAL LETTER:

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA.

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho, MS Ortho, DNB Ortho
Chairperson, Research Committee & Principal

Dr. Nihal Thomas,
MD., MNAMS., DNB (Endo), FRACP (Endo), FRCP (Edin), FRCP (Glasg)
Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

September 2, 2015

Dr. Saraswathi Ramanathan
Department of PMR
Christian Medical College,
Vellore 632 004

Sub: **Fluid Research Grant Project:**
Comparison of standard outpatient screening tools and nerve conduction studies for the diagnosis of diabetic peripheral neuropathy – A Pilot study.
Dr. Saraswathi Ramanathan, Emp. No: 21074, Dr. Raji Thomas, Dr. Asem Rangita Chanu, Emp. No: 33499, Dr. Prasanth Chalagiri, Emp. No: 20777 Dept. of PMR, Dr. Nihal Thomas, Emp. No: 13132, Dr. Dukhbandu Naik, Emp. No: 31624, Dr. Mahesh, Emp. No:20815, Dept. of Endocrinology, Diabetes & Metabolism

Ref: IRB Min No: 9468 [OBSERV] dated 05.06.2015

Dear Dr. Saraswathi Ramanathan,

I enclose the following documents:-

1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Nihal Thomas, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

B. J. Prashantham

Dr. BJ Prashantham
Chairperson (Ethics Committee),
Institutional Review Board

Cc: Dr. Raji Thomas, Department of PMR, CMC

1 of 5

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA.

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
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September 2, 2015

Dr. Saraswathi Ramanathan
Department of PMR
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Ref: IRB Min No: 9468 [OBSERV] dated 05.06.2015

Dear Dr. Saraswathi Ramanathan,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "Comparison of standard outpatient screening tools and nerve conduction studies for the diagnosis of diabetic peripheral neuropathy – A Pilot study" on June 05th 2015.

The Committee reviewed the following documents:

1. IRB Application format
2. Proforma
3. Michigan neuropathy screening instrument
4. Patient Information Sheet and Informed Consent Form (English, Tamil, Hindi)
5. Cvs of Dr. Raji Thomas, Nihal Thomas, Dukhabandhu Naik, Mahesh, Prashanth H Chalageri, Asem Rangita Chanu, Saraswathi Ramanathan
6. No. of documents 1- 4

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on June 05th 2015 in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore 632 002. 2 of 5

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA.

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho, MS Ortho, DNB Ortho
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Dr. Nihal Thomas,
MD., MNAMS., DNB (Endo), FRACP (Endo), FRCP (Edin), FRCP (Glasg)
Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

Fluid Grant Allocation:

A sum of 50,000/- INR (Rupees Fifty thousand) will be granted for 1 years and out of which a maximum of Rs.5000 can be spent for stationery, printing, Xeroxing and computer charges (if computers used are within the institution).

Yours sincerely

B. J. Prashantham

Dr. BJ Prashantham
Chairperson (Ethics Committee),
Institutional Review Board

NOT TO BE MINISTERED UNTO BUT TO MINISTER
CHRISTIAN MEDICAL COLLEGE
VELLORE
INDIA

Cc: Dr. Raji Thomas, Department of PMR, CMC

ANNEXURE 3: PATIENT INFORMATION SHEET:

A study is being done to correlate the clinical methods and nerve conduction studies in early diagnosis of diabetic peripheral neuropathy. You are being invited to participate in this research study. Before you make a decision, we would like to provide certain details about the research which are essential for you to know.

1. WHAT IS THE PURPOSE OF THIS STUDY?

Diabetes is a worldwide problem and is very common in our country. It is a condition wherein the blood sugar levels are high. As the duration of disease increases, it is known to produce various complications, that is, it can affect the eyes, kidneys, heart, nerves etc. When it affects the nerves, we get a condition called diabetic neuropathy. This is an important complication and patients need to be aware of it because it can eventually lead to foot ulcers, amputation (removal of a part of the body), gangrene and even death. The aim of this study is to study the various clinical methods and nerve conduction studies in early detection of diabetic neuropathy. Every patient who is diagnosed with Diabetes mellitus will be given a questionnaire to enquire about the symptoms of neuropathy. Then a quick clinical examination will be done. Then the sense of vibration will be tested using a biothesiometer (a hand held vibrating instrument which will be placed at the tip of great toe of the patient). Then they will all be subjected to nerve conduction studies (a test used to evaluate the function of nerves and speed at which the nerve conducts). After that, a comparison of all these tests will be done to see which picks up the disease earlier and helps in early detection.

2. WHO WILL PERFORM THE TEST? WHAT ARE THE POSSIBLE COMPLICATIONS?

The examination and the nerve conduction studies will be performed by the principle investigator. There is no problem in performing this test. The investigation will not adversely affect my health in any forseeable manner. There

may be a small tolerable pain while performing this test. But it usually resolves within minutes of completion of the study.

3. WILL WITHDRAWL OR NON PARTICIPATION AFFECT THE USUAL TREATMENT?

The patient can withdraw from the study at any point of time without being obliged to give a reason. He is also free to decide regarding his participation. Non-participation or withdrawal will not affect his usual treatment at any point of time as an out-patient or an in-patient.

4. DO I HAVE TO PAY FOR THE INVESTIGATIONS?

You will not have to pay for the nerve conduction studies being done as a part of the research.

5. WILL MY TEST REPORTS BE KEPT CONFIDENTIAL?

The results of the study will be published in a medical journal but you will not be identified by any name in any public presentation of results. However your medical notes may be reviewed by people associated with the study, without any additional permission, should you decide to participate in the study.

For any further questions, contact: Dr. Saraswathi Ramanathan- 09791102971

ANNEXURE 4: INFORMED CONSENT FORM TO PARTICIPATE IN A RESEARCH STUDY

Study Title: CO-RELATION OF CLINICAL TESTING AND NERVE CONDUCTION STUDIES IN DIABETIC PERIPHERAL NEUROPATHY
Study Number: _____

Subject's Name: _____

Date of Birth / Age: _____

(Subject)

(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ]

(ii) I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ]

(iii) I understand that the Sponsor of the clinical trial, others working on the Sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published. [ ]

(iv) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). [ ]

(v) I agree to take part in the above study. [ ]

Signature (or Thumb impression) of the Subject/Legally Acceptable

Date: _____/_____/_____

Signatory's Name: _____ Signature:

Signature of the Investigator: _____

Date: _____/_____/_____

Study Investigator's Name: _____

Signature or thumb impression of the Witness: _____

Date: _____/_____/_____

Name & Address of the Witness: _____

ANNEXURE 5: PROFORMA FOR DATA COLLECTION

Patient Details:

Name of the patient –

Age (years)– Sex – Male / Female

Occupation –

Education –

Address –

Hospital no. –

Contact no. –

Presenting complaints :

History of Diabetes Mellitus-

Year of diagnosis/ age at onset –

Type of Diabetes –

Initial presenting symptoms-

Initial GRBS/AC/PC –

Treatment history- Insulin –
OAD -
Current treatment regime -

Foot History

Vascular symptoms

- Claudication – Y/ N
- Rest pain – Y/ N
- Non healing ulcer – Y/ N

Footwear

- Type of footwear MCR – Y/ N
  Normal – Y/ N

- Barefoot walking- Y/N
- Walking hours per day
- Standing hours per day

Complications of Diabetes Mellitus :

Diabetic Retinopathy

- Yes [ ]
- No [ ]

Diabetic Nephropathy

- Yes [ ]
- No [ ]

Diabetic Neuropathy

- Yes [ ]
- No [ ]

Cardiovascular disease

- Yes [ ]
- No [ ]

Cerebrovascular disease

- Yes [ ]
- No [ ]

Peripheral vascular disease

- Yes [ ]
- No [ ]

Past History:

- Diabetes mellitus
- Hypertension
- Foot ulcer

- Tia / stroke
- Others

Personal History :

Smoking –

Alcohol consumption -

Height –

Weight –

Body Mass Index –

SYSTEMIC EXAMINATION

Respiratory system –

Cardiovascular system –

Abdomen –

Central nervous system –

INVESTIGATIONS –

Hemoglogin –

Glycoylated haemoglobin –

Serum fasting Lipid profile –

Serum Creatinine –

Urine Microalbumin –

ANNEXURE 6: MICHIGAN NEUROPATHY SCREENING INSTRUMENT

A. History (To be completed by the person with diabetes)

Please take a few minutes to answer the following questions about the feeling in your legs and feet. Check yes or no based on how you usually feel. Thank you.

1. Are you legs and/or feet numb? Yes No
2. Do you ever have any burning pain in your legs and/or feet? Yes No
3. Are your feet too sensitive to touch? Yes No
4. Do you get muscle cramps in your legs and/or feet? Yes No
5. Do you ever have any prickling feelings in your legs or feet? Yes No
6. Does it hurt when the bed covers touch your skin? Yes No
7. When you get into the tub or shower, are you able to tell the hot water from the cold water? Yes No
8. Have you ever had an open sore on your foot? Yes No
9. Has your doctor ever told you that you have diabetic neuropathy? Yes No
10. Do you feel weak all over most of the time? Yes No
11. Are your symptoms worse at night? Yes No
12. Do your legs hurt when you walk? Yes No
13. Are you able to sense your feet when you walk? Yes No
14. Is the skin on your feet so dry that it cracks open? Yes No
15. Have you ever had an amputation? Yes No

Total:

MICHIGAN NEUROPATHY SCREENING INSTRUMENT

B. Physical Assessment (To be completed by health professional)

1. Appearance of Feet

	Right	Left
a. Normal	☐ 0 Yes ☐ 1 No	Normal ☐ 0 Yes ☐ 1 No
b. If no, check all that apply:		If no, check all that apply:
Deformities	☐	Deformities ☐
Dry skin, callus	☐	Dry skin, callus ☐
Infection	☐	Infection ☐
Fissure	☐	Fissure ☐
Other	☐	Other ☐
specify:	_____	specify: _____

	Right		Left	
	Absent	Present	Absent	Present
2. Ulceration	☐ 0	☐ 1	☐ 0	☐ 1

	Right			Left		
	Present	Present/ Reinforcement	Absent	Present	Present/ Reinforcement	Absent
3. Ankle Reflexes	☐ 0	☐ 0.5	☐ 1	☐ 0	☐ 0.5	☐ 1
	Present	Decreased	Absent	Present	Decreased	Absent
4. Vibration perception at great toe	☐ 0	☐ 0.5	☐ 1	☐ 0	☐ 0.5	☐ 1
5. Monofilament	Normal	Reduced	Absent	Normal	Reduced	Absent
	☐ 0	☐ 0.5	☐ 1	☐ 0	☐ 0.5	☐ 1

Signature:_____ Total Score _____ /10 Points

ANNEXURE 7: NERVE CONDUCTION STUDIES METHODOLOGY:

Sural sensory nerve conduction:

The active electrode was placed between the lateral malleolus and the tendoachilles. The reference electrode was placed 3cm distal to the active electrode. The ground was placed between the recording and the stimulating electrodes. Stimulation was done at the posterior midline of the leg just beneath the prominence of gastrosoleus, anode was 3 cms proximal to the cathode.

Superficial peroneal sensory nerve conduction:

The active electrode was placed over the dorsum of the foot at the level of the malleoli, slightly lateral to the midline. Reference electrode was placed 3cms distal to the active electrode. Ground was placed at the distal dorsum of leg between the recording and the stimulating electrodes. Stimulation was done over the distal anterolateral leg around 10-14cms proximal to the lateral malleolus. Anode was 3cms proximal to the cathode.

Tibial nerve conduction:

The active electrode was placed over the belly of the abductor hallucis on the medial aspect of the foot, just beneath the navicular bone. The reference electrode was placed 3cms distal to the active electrode, over the tendon of the abductor hallucis. Ground electrode was placed over the dorsum of the foot between the active and reference electrodes. Stimulation at the ankle was done posterior to the medial malleolus with the

anode 3cms proximal to the cathodeStimulation at the knee was done in the popliteal fossa, one to two finger breadths medial to the biceps femoris tendon.

Common peroneal nerve conduction:

The active electrode was placed over the dorsum of the footover the belly of the EDB muscle. The reference electrode was placed 3cms distal to the active electrode over the tendon of the EDB. Ground was placed on the dorsum of the foot between the active and reference electrode. Distal stimulation was done over the anterior ankle with anode 3cms distal to the cathode. Proximal stimulation was done just below the head of fibula and in the lateral aspect of the popliteal fossa.

Median Sensory nerve conduction:

This was done using ring electrodes. The active electrode was placed around the mid-portion of the proximal phalynx of the second digit (just proximal to the proximal interphalyngeal joint). Reference electrode was placed 3cms distal to the active electrode. Ground was placed on the dorsum of the hand between the active and the reference electrodes. Stimulation was done over the median nerve between the FCR(Flexor carpii radialis) and the palmaris longus, around 14cms proximal to the recording electrode. Anode was 3cms proximal to the cathode.

Radial Sensory nerve conduction: This was done using disc electrodes. The active electrode was placed over the anatomical snuff box formed by the extensor pollicis brevis

and the abductor pollicis longus tendons laterally and extensor pollicis longus tendon medially. The reference electrode was placed 3cms distal to the active electrode. Ground electrode was placed over the dorsum of the hand between the recording and stimulating electrodes. Stimulation was done over the dorsolateral edge of the radius bone, 10cms from the active recording electrode with the anode 3cms proximal to the cathode.

Median Motor nerve conduction:

The active electrode was placed over the belly of the abductor pollicis brevis (APB) muscle and the reference electrode was placed 3cms proximal to the active electrode, over the tendon of the APB muscle, at the base of the proximal phalynx of the thumb. . Ground electrode was placed on the dorsum of the hand between te active and the reference electrodes. Stimulation at the wrist was done 2-3 cms proximal to the distal crease between the FCR and Palmaris longus. At the elbow, stimulation was done at the antecubital fossa over the brachial artery, medial to the biceps tendon. At the arm, stimulation was done at the upper arm, just below the belly of the biceps. Anode was proximal to the cathode.

Ulnar motor nerve conduction studies:

The active electrode was placed over the belly of the ADM (abductor digiti minimi) and the reference electrode was placed over the tendon of ADM at the base of the proximal phalynx of little finger. Ground is placed on the dorsum of the hand, between the recording and stimulating electrodes. Stimulation at the wrist was done 2-3cms proximal

to the distal crease, just lateral to the flexor carpi ulnaris (FCU) tendon with the anode 3cm proximal to the cathode. Below elbow stimulation was done with the cathode 3-4cms distal to the medial epicondyle and retrocondylar groove. Above elbow stimulation was done at 4-5cms proximal to the medial epicondyle and retrocondylar groove between the biceps and the triceps. with the anode 3cms proximal to the cathode.

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