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Ministry of Higher Education
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College of Medicine



**A STUDY OF PHYSIOLOGICAL AND CLINICAL
CHANGES IN PATIENTS PRESENTED WITH
DIABETIC PERIPHERAL NEUROPATHY**

A Thesis

Submitted to the Council of College of Medicine
University of Babylon in Partial Fulfillment of the
Requirements for the Degree of Master of Science in
Medical Physiology

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Dedication

بسم الله الرحمن الرحيم

To my family

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دراسة التغيرات الفسلجية والسريرية في المرضى الذين يعانون من اعتلال الأعصاب المحيطية المصاحب لمرض السكري

رسالة مقدمة الى

كلية الطب/ جامعة بابل

وهي جزء من متطلبات نيل درجة ماجستير علوم في علم الفسلجة الطبية

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وَمَنْ يَتَوَكَّلْ عَلَى اللَّهِ فَهُوَ حَسْبُهُ إِنَّ

اللَّهُ بَالِغُ أَمْرِهِ قَدْ جَعَلَ اللَّهُ لِكُلِّ

شَيْءٍ قَدْرًا

صدق الله العلي العظيم

سورة الطلاق- الآية (3)

الخلاصة

تضمنت هذه الدراسة معرفة التغيرات التي تحدث لبعض المعايير الطبية والكيموحيوية والفسلجية الكهربائية في مرضى السكري المصابين باعتلال الأعصاب وذلك لأهمية هذه المعايير في تشخيص، تحديد، معرفة بعض عوامل خطوره الممكنة و تقدير استجابة المريض للعلاج.

استمرت الدراسة من تشرين الأول 2008 حتى أيار 2009. كان العدد الكلي لمجموعة الدراسة 225 شخصاً تم تقسيمهم الى 105 مريض و 120 شخص في مجموعة السيطرة. تم تصنيف المرضى إلى مجموعتين اعتماداً على نوع السكري المصابين به: المرضى المصابين بداء السكري من النوع الأول (35 مريض) تراوح معدل أعمارهم بين 16-34 سنة و المرضى المصابين بالسكري من النوع الثاني (70 مريض) تراوح معدل أعمارهم بين 45-72 سنة. أما مجموعة السيطرة فقد تم تقسيمهم إلى مرضى مصابين بداء السكري وغير مصابين باعتلال الأعصاب (60 مريض) تراوح معدل أعمارهم بين 18-52 سنة ومجموعة الأشخاص الأصحاء (60 شخص) تراوح معدل أعمارهم بين 18-70 سنة. المرضى مسجلين في مركز السكري في مستشفى مرجان التعليمي ولديهم زيارات شهرية الى المركز. تم فحص المرضى ومجموعة السيطرة من قبل طبيب اختصاص.

أخضع المرضى ومجموعة السيطرة المنخرطين في الدراسة الى تقييم شامل تضمن: التقييم السريري (التاريخ المرضي و الفحص السريري)، التقييم الكيموحيوي (قياس الجلوكوز في الدم، مجموعة الدهون، السيلينيوم وفيتامين E في مصل الدم)، قياس مستوى الهيموغلوبين الجلوكوزي و التقييم الفسلجي الكهربائي المتضمن دراسة توصيل الأعصاب الحسية والحركية.

و قد وجدت الدراسة بأن الخدر والتئمل كانا من اكثر اعراض اعتلال الأعصاب شيوعاً متبوعاً بالألم و ضعف الأعصاب الحركية.

في حين كان من اول العلامات المرضيه شيوعاً وأكثرها حدوثاً هو انخفاض احساس الإهتزاز (vibration perception) في الأطراف يتبعها انخفاض المنعكس الحسي للأوتار (tendon reflexes) للأطراف السفلى يتبعها ضعف الإحساس لوخز الأبر والإحساس الحراري وأخيراً ضعف الأعصاب الحركية. كذلك وجدت الدراسة ان مستوى السكر والهيموغلوبين الجلوكوزي في الدم اعلى في المرضى من مجموعة السيطرة مع فروقات معنوية ($P < 0.05$). كان مستوى مجموعة الدهون في الدم اعلى في المرضى من مجموعة السيطرة بدون فروقات معنوية. كذلك وجدت الدراسة وجود

فروقات معنوية في مستوى السيلينيوم ($P<0.05$) و فيتامين E ($P<0.05$) في مصل الدم بين المرضى ومجموعة السيطرة.

اما فيما يخص التغيرات الفسلجية الكهربائية وجدت الدراسة زيادة في الكمون الحركي والحسي (motor & sensory latency) بين المرضى ومجموعة السيطرة مع فروقات معنوية ($p<0.05$) كذلك وجود انخفاض في سرعة التوصيل للأعصاب الحركية والحسية (conduction velocity) والقمة الحركية والحسية (Amplitude) بين المرضى ومجموعة السيطرة مع فروقات معنوية.

وجدت الدراسة بأن ارتفاع الغلوكوز في الدم بسبب نقص او مقاومة العمل الأيضي لهرمون الإنسولين هو من اهم عوامل الخطورة التي يمكن تعديلها لمرض اعتلال الأعصاب بالإضافة الى ذلك فان الجهد التأكسدي يلعب دوراً مهماً.

CHAPTER ONE

Introduction

1 Introduction:-

Diabetes Mellitus (DM) is a clinical syndrome characterized by hyperglycemia due to absolute or relative insulin deficiency (Davidson's, 2006). There are 2 types of complications of diabetes mellitus:- macrovascular and microvascular complications. The macrovascular complications include: - cerebrovascular, cardiovascular and peripheral vascular diseases. The microvascular complications include nephropathy, retinopathy and neuropathy and it result from chronic hyperglycemia (Aaberg *et al.*, 2008). Diabetic neuropathy is the most common chronic complication affecting both type 1 and type 2 diabetic patients (Sima, 2004). The prevalence of diabetic neuropathy varies from 10% within 1 year of diagnosis to 50% in patients with diabetes for 25 years or longer. The average prevalence is 30% (Tesfaye, 2006). Distal symmetric sensorimotor peripheral neuropathy (DPN) is by far the most common type of diabetic neuropathy, typically presented as a slowly progressive primarily sensory deficit in a length-dependent fashion, with symptoms starting in the feet and spreading upwards, evoking the classic stocking glove distribution (Tracy and Dyck, 2008). The signs and symptoms of DPN vary depending on fiber type involved. With large fiber disease impairing proprioception and light touch. Small fiber disease impairs pain and temperature perception, leading to paresthesias, dysesthesias, and/or neuropathic pain. Distal weakness occurs only in the most severe cases. Diabetic peripheral neuropathy might be presented early with diminished or absent deep-tendon reflexes, particularly the Achilles tendon reflex often indicates mild and otherwise

asymptomatic DPN. More advanced neuropathy may be firstly presented with late complications such as ulceration or neuroarthropathy (Charcot's joints) of the foot (Yagihashi *et al.*, 2007). Hyperglycemia clearly plays a key role in the development and progression of diabetic neuropathy as well as the other microvascular complications of diabetes. Long term hyperglycemia elicits enhanced polyol pathway, increased nonenzymatic glycation of structural proteins, increased oxidative stress as well as altered protein kinase C activity and poly ADP-ribose polymerase (PARP) activation that are all interrelated for the cause and development of neuropathy. It is yet to be clear, however, that these mechanisms are related to each other or independently operate (Edward's *et al.*, 2008). Apart from direct hyperglycemia-induced damage, ischemia caused indirectly from decrease in neurovascular flow almost certainly plays a role (Misawa *et al.*, 2004). The duration of diabetes and degree of metabolic control are the two major risk factors of the development of neuropathy and determinant of its severity. Other factors, such as patient's age, sex, type of DM, height, lipid profile abnormalities, and presence of proliferative retinopathy, nephropathy, and cardiovascular diseases, also have been implicated (Harati, 2007). Diabetic peripheral neuropathy can be diagnosed by variety of ways including:- full history and neurological examination, nerve biopsy and electrophysiological study (nerve conduction study) which shows a pattern of abnormality that reflects the pathological process of DPN. The electrophysiological changes include prolongation of latency (sensory and motor), decrease amplitude & decrease conduction velocity. These changes are elicited first in the sensory nerves of lower limbs (Kimura, 2001).

1.1 Aims of study:-

This study aims to:

- 1- Identify some possible risk factors of diabetic peripheral neuropathy in Babylon province.
- 2- Assessment of patients with diabetic polyneuropathy that includes:-
 - a- Clinical assessment, that includes taking full history and examination.
 - b- Measurement of glycosylated hemoglobin (HbA1c).
 - c- Biochemical assessment, include measurement of blood sugar, lipid profile, selenium and vitamin E measurement.
 - d- Electrophysiological assessment, by doing nerve conduction study (motor and sensory).

CHAPTER TWO

Literature Review

2.1 Diabetes Mellitus (DM):-

Is a syndrome of impaired carbohydrate, fat, and protein metabolism caused by either lack of insulin secretion or decreased sensitivity of the tissues to insulin (Guyton and Hall, 2006).

2.2 Types of diabetes:-

There are 2 major types of DM:

1-Type 1 DM: is also called insulin requirement DM and caused by lack of insulin secretion.

2-Type 2 DM: is caused by decreased sensitivity of target tissues to the metabolic effects of insulin. This reduced sensitivity to insulin is called insulin resistance (Davidson's, 2006). In both types of diabetes mellitus, insulin lack or insulin resistance will result in reduced entry of glucose into the cells (decreased peripheral utilization) which will results in metabolic derangement. In the absence of insulin action, the entry of glucose into skeletal, cardiac, smooth muscle and other tissues is decreased. Intestinal absorption of glucose is unaffected, as is its reabsorption from the urine by the cells of the proximal tubules of the kidneys. Glucose uptake by most of the brain and the red blood cells is also normal (Ganong, 2005).

2.2.1 Type 1 Diabetes:-

Is lack of insulin production by beta cells of pancreas. It might result from injury to the beta cells of the pancreas or diseases that impair insulin

production. Viral infections or autoimmune disorders may be involved in the destruction of beta cells in the islets of Langerhan's in the pancreas in many patients with Type 1 diabetes, although heredity also plays a major role in determining the susceptibility of the beta cells to destruction by these insults. In some instances, there may be a hereditary tendency for beta cell degeneration even without viral infections or autoimmune disorders. The usual onset of Type 1 diabetes occurs at about 14 years of age (Guyton and Hall, 2006).

2.2.2 Type 2 Diabetes:-

Type 2 diabetes is far more common than Type 1, accounting for about 90 per cent of all cases of diabetes mellitus. In most cases, the onset of Type 2 diabetes occurs after age 30 years, often between the ages of 50 and 60 years, and the disease develops gradually. Therefore, this syndrome is often referred to as adult-onset diabetes mellitus (Guyton and Hall, 2006).

Obesity, insulin resistance, and metabolic syndrome usually precede development of Type 2 diabetes. Type 2 diabetes, in contrast to Type 1, is associated with increased plasma insulin concentration (hyperinsulinemia). This occurs as a compensatory response by the pancreatic beta cells for diminished sensitivity of target tissues to the metabolic effects of insulin, a condition referred to as insulin resistance. The decrease in insulin sensitivity impairs carbohydrate utilization and storage, raising blood glucose and stimulating a compensatory increase in insulin secretion (Aaberg *et al.*, 2008).

2.3 Epidemiology of diabetes mellitus:-

Diabetes is world-wide in distribution and the incidence of both types is rising. However the prevalence of both varies considerably in different parts

of world and this is probably due to differences in genetic and environmental factors. Diabetes affects 1–2% of the population worldwide (Davidson's, 2006). The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 (Girach, and Vignati, 2006).

2.4 Complications of DM:-

There are 2 types of complications of DM:

Microvascular and macrovascular. Micro vascular complications include nephropathy, retinopathy and neuropathy. While macrovascular complications include cerebrovascular, peripheral vascular and cardiovascular diseases (Mundet *et al.*, 2008).

2.4.1 Micro vascular complications:-

The microvascular complications of both Type 1 and Type 2 DM results from chronic hyperglycemia. Clinical trials involving large numbers of individuals with Type 1 or Type 2 DM have conclusively demonstrated that a reduction in chronic hyperglycemia prevents or reduces the incidence or severity of microvascular complications. There are other incompletely defined factors that modulate the development of microvascular complications. For example, oxidative stress, loss of neurotrophic support, autoimmune mechanism, C-peptide deficiency, and others (Weiswasser *et al.*, 2003). Despite longstanding DM, some individuals never develop microvascular complications. Many of these patients have glycemic control that is indistinguishable from those who develop microvascular complications. Because of these observations, it is suspected that a genetic susceptibility for developing particular complications exists. However, the genetic loci responsible for these susceptibilities have not yet been identified

(Harrison's, 2002). In developed countries, diabetic retinopathy is the leading cause of acquired blindness in adults, and diabetic nephropathy is the second cause of renal replacement therapy. Peripheral neuropathy interacts with macrovascular disease and infection in the pathophysiology of diabetic foot, increasing the risk of foot ulcers and amputations (Cardoso and Salles, 2008). Diabetic neuropathy is the leading cause of non traumatic limb amputation (Edwards *et al.*, 2008).

2.4.1.1 Nephropathy:-

Diabetes is the most common cause of end-stage renal disease (ESRD). It accounts for 37% of cases of ESRD in United States (Davidson's, 2006). The course of diabetic nephropathy is slow; the first clinical evidence is an increase in urinary albumin excretion (microalbuminuria), which develops into overt nephropathy (proteinuria) in 20–40% of patients with Type 2 diabetes. About 20% of patients (Type 2 diabetics) will have progressed to ESRD within 20 years after onset of overt nephropathy. In patients with Type 1 diabetes with overt nephropathy, ESRD develops in 50% of patients within 10 years and in >75% by 20 years (Leiter, 2005).

2.4.1.2 Retinopathy:-

Diabetic retinopathy is the leading cause of blindness in working-age adults. The majority of cases of blindness due to diabetic retinopathy are thought to be preventable (Ahmed, 2005). The incidence of retinopathy increases with duration of diabetes. Indeed, >60% of patients with Type 2 diabetes and most patients with Type 1 diabetes will have retinopathy in the first 20 years of the disease. Endothelial dysfunction plays a major role in the development of diabetic retinopathy (Wirotstiko *et al.*, 2008).

2.4.1.3 Neuropathy:-

Diabetic neuropathy is the most common complication of DM, it is caused by hyperglycemia and subsequent metabolic derangement, with prevalence of 50%. The prevalence of neuropathy is directly related to the duration of DM and degree of metabolic control (Edward's *et al.*, 2008).

2.5 Diabetic Neuropathy:-

2.5.1 Definition:-

Diabetic neuropathy is a descriptive term that encompasses a spectrum of clinical and subclinical syndromes with differing anatomical distributions, clinical courses and possibly differing underlying pathogenetic mechanisms. Each is characterized by diffuse or focal damage to peripheral somatic or autonomic nerve fibers resulting from diabetes mellitus, although indistinguishable syndromes may occur idiopathically or in association with other disorders in non diabetic individuals (Sima, 2004).

2.5.2 Epidemiology:-

Diabetic neuropathy is one of the most common complications of diabetes and the most common form of neuropathy in the developed world (Tesfaye, 2006). The prevalence of neuropathy is estimated to be about 8% in newly diagnosed patients (at time of diagnosis) and greater than 50% in patients with long-standing disease (Boulton *et al.*, 2005). The prevalence is similar in males and females (Tesfaye, 2006). Diabetic neuropathy accompanying Type 1 diabetes occurs more predictably and progresses more rapidly resulting in a more severe neuropathy. Close to 100% of Type 1 patients eventually develop neuropathy (Sima, 2004). Most patients with

diabetic neuropathy are asymptomatic. Dyck and colleagues, (1999) evaluated 380 patients in a cohort of diabetic patients representative of the community and found out that 66% of the patients with Type 1 and 59% of the patients with Type 2 DM had some type of neuropathy. Despite the finding that diabetic neuropathy occurs commonly, most cases were asymptomatic and detected because of findings on clinical examination and electrophysiologic studies.

2.5.3 Classification of diabetic neuropathy:-

The association between diabetes mellitus (DM) and neuropathy has been recognized for over 100 years and soon it was realized that different subtypes existed, and so the first classification was proposed by Leyden in 1893, with hyperesthetic (painful), paralytic (motor), and ataxic forms of diabetic neuropathy. There are many varieties of neuropathy classified under the term diabetic neuropathy, some are clearly linked to hyperglycemia and subsequent metabolic and ischemic changes, others are associated with compressive etiologies, but still others are associated with inflammatory/immune processes (Weiswasser *et al.*, 2003). The most common of these syndromes is diabetic sensorimotor peripheral neuropathy (DPN) and is generally what is referred to as diabetic neuropathy in the generalized clinical setting. The way of classification preferred here is to divide diabetic neuropathies by the clinical pattern into symmetrical or asymmetrical form (Edward's *et al.*, 2008) as in the following box (2.1).

Box 2.1 *Neuropathies associated with diabetes mellitus based on clinical pattern* (Tracy and Dyck, 2008).

Symmetric

- .Diabetic peripheral neuropathy (DPN)
- .Diabetic autonomic neuropathy (DAN)
- .Painful sensory neuropathy with weight loss (diabetic cachexia)
- Insulin neuritis
- .Hypoglycemic neuropathy
- .Chronic inflammatory demyelinating polyradiculoneuropathy in DM

Asymmetric

- .Diabetic cranial neuropathy
- .Diabetic mononeuropathy:
 - .Median neuropathy at the wrist
 - .Ulnar neuropathy at the elbow
 - .Peroneal neuropathy at the fibular head
- .Diabetic radiculoplexus neuropathies (DRPN)
- .Diabetic thoracic radiculoneuropathy (DTRN)
- .Diabetic lumbosacral radiculoplexus neuropathy (DLRPN)
- .Diabetic cervical radiculoplexus neuropathy (DCRPN)

2.5.4 Diabetic peripheral (sensorimotor) neuropathy (DPN):-

Diabetic symmetric sensorimotor peripheral neuropathy is by far the most common type of diabetic neuropathy. The syndrome has an insidious onset and typically affects the most distal extremities first, resulting in a stocking pattern of sensory loss. The toes are affected first; the process then extends up the legs, and only later are the upper extremities involved, beginning with the fingertips. In very severe cases, the disease may also affect the anterior portions of the chest and abdomen (Obrosova, 2008). There are two broad categories of sensory symptoms of DPN, positive and negative. Positive symptoms include pain, paresthesias and aberrant, exaggerated sensitivity to normally painless or moderately painful stimuli (allodynia and hyperalgesia). Negative symptoms consist of loss of sensory perception in one or several modalities. Motor symptoms, if present, are manifest as muscle weakness, and thus they are also negative (Dobretsov *et al.*, 2007). Myelinated and unmyelinated small-fibers as well as myelinated large sensory fibers are typically involved, although recent evidence suggests that the earliest manifestations are primarily small-fiber type. The small-fiber sensory loss results in loss of pain and temperature sensation, which may predispose the patient to injury and possible foot ulceration. Earlier manifestations of large-fiber neuropathy include abnormal nerve conduction velocities, later results in loss of vibration and proprioceptive sensation, leading to sensory ataxia in severe cases (Vinik *et al.*, 2008). Types of nerve fibers are shown in box 2.2 (Ganong, 2005).

Box 2.2 shows the General Classification of nerve fibers (Ganong, 2005)

Type	Function	Conduction velocity (m/s)	properties
A α	Proprioception, somatic, motor	70-120	Large myelinated
A β	Touch, pressure, motor	30-70	Large myelinated
A γ	Motor to muscle spindle	15-30	Medium myelinated
A δ	Pain, touch cold	12-30	Medium myelinated
C	Pain, temperature	0.5-2	Small unmyelinated

Motor involvement is usually minor and restricted to the distal extremities and abnormally redistributes weight bearing, contributing to callus and ulcer formation. Reflexes may be diminished and ankle jerks are frequently dropped (Tesfaye, 2006). Early, DPN affects the distal parts of extremities and this might be a manifestation of dying back degeneration, with the primary insult consisting of the impairment of synthesis or efferent axonal transport of proteins, therefore affecting the function of the longest axons in the body that are most dependent on these mechanisms. Failure of protein synthesis, including synthesis of some important neurotrophic molecules, in diabetes could result in impaired nerve regeneration and dying back axonopathy (Karsidag *et al.*, 2005). Alternatively, accumulation of the effects of multiple injuries randomly located along the axon, for example demyelinating injuries, may result in a clinical picture that is practically indistinguishable from dying back neuropathy. The longest axons in the body will most likely be hit by a critical number of such local insults and their function will fail first. Peripheral axons are the longest in the human body, and this might be an important factor explaining peripheral nervous system (PNS) involvement superseding the central nervous system complications. Another potentially important reason for the high vulnerability of PNS to diabetic injury is the relatively weak anti-oxidative defenses of peripheral neurons (Dobretsov *et al.*, 2007). Most patients with distal symmetric sensorimotor peripheral neuropathy are asymptomatic or mildly symptomatic, and the syndrome may only be detected with careful physical examination (Rubino *et al.*, 2007).

2.5.5 Pathology and Pathogenesis of diabetic peripheral neuropathy:-

The pathophysiology of diabetic neuropathy has not been well understood, and presumably includes a complex interplay between metabolic abnormalities directly related to hyperglycemia and structural nerve damage caused by microangiopathy (Misawa *et al.*, 2004). The main pathogenetic mechanisms are: increase activity of polyol pathway, increase production of advanced glycation end products, loss of neurotrophic support, activation of protein kinase C isomer, oxidative stress, microvascular insufficiency, immune mechanism, C-peptide deficiency (park *et al.*, 2004) and others as shown in Figure 2.1 and Box 2.3:

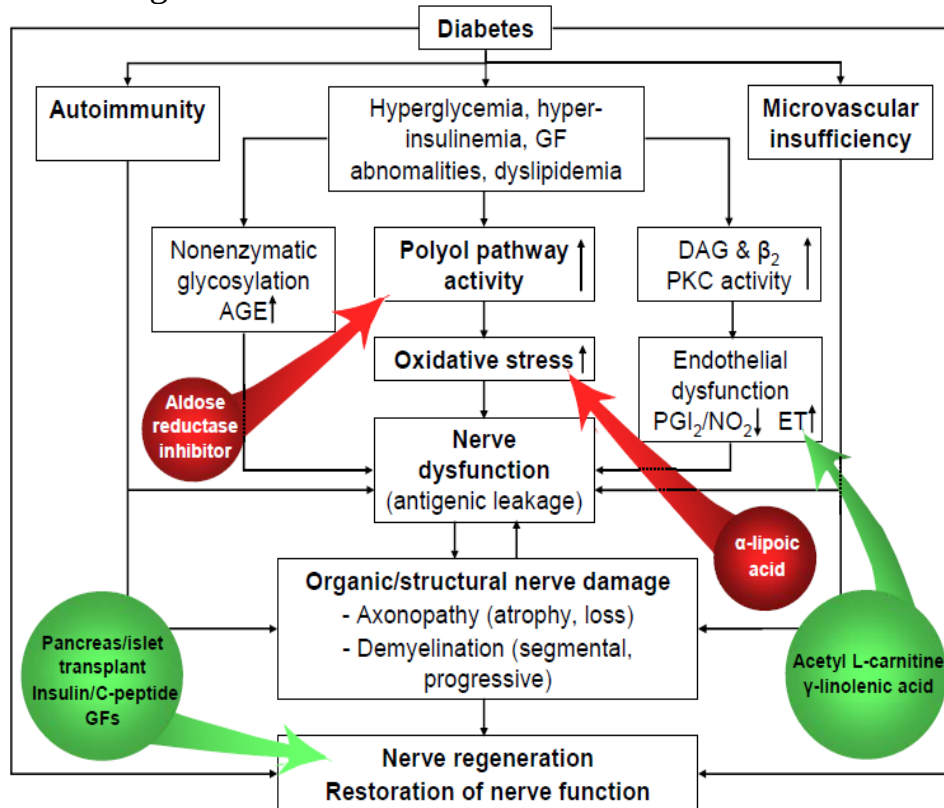


Figure 2.1 The important pathophysiological change of Diabetic peripheral neuropathy with its therapeutic intervention (Boucek, 2006).

GF=growth factor, AGE=advanced glycation end product, DAG=diacyl glycerol, PKC=protein kinase C, PGI=prostaglandin, NO=nitric oxide.

Box 2.3 *Proposed abnormalities implicated in the pathogenesis of diabetic peripheral neuropathy (Harati, 2007).*

1-Abnormalities that suggest a vascular etiology

- Advanced glycation of vessel wall
- Endothelial cell swelling and pericyte degeneration
- Multifocal ischemic proximal nerve lesions
- Increased oxygen free radical activities
- Reduced endothelial nitric oxide activity

2- Abnormalities that suggest a metabolic etiology

- Accumulation of sorbitol
- Reduction in the rate of synthesis and transport of intra-axonal proteins
- Reduction in nerve sodium-potassium-ATPase
- Enhanced protein kinase C activity
- Reduced incorporation into myelin of glycolipids and amino acids
- Reduced nerve L-carnitine level
- Abnormal inositol lipid metabolism
- Impaired essential fatty acid and prostaglandin metabolism

3- Other abnormalities

- Increased nerve edema
- Increased blood-nerve permeability
- Impaired endogenous neurotrophic or vascular growth factors
- Insulin deficiency

2.5.5.1 Polyol pathway:-

Polyol pathway is a simple metabolic pathway converting glucose to sorbitol by enzyme aldose reductase (AR), then converting sorbitol to fructose by sorbitol dehydrogenase (SDH) (Yagihashi *et al.*, 2007). Hyperglycemia induces the hyperactivation of the polyol pathway and the excessive nerve accumulation of sorbitol and fructose. These abnormalities under diabetes lead to nonenzymatic glycation, oxidative injury, and hypoxia and so on (Shimoshige *et al.*, 2009). Enhanced polyol pathway also causes depletion of the myoinositol pool in peripheral nerve (important in signal transduction) and depletion of taurine (an antioxidant) (Sima *et al.*, 2004). Since nicotinamide adenine dinucleotide (NADPH) is consumed by aldose reductase-mediated reduction of glucose to sorbitol and NADPH is required for regeneration of reduced glutathione (GSH), this too contributes to oxidative stress (Tracy and Dyck, 2008).

2.5.5.2 Protein kinase C pathway (PKC):-

The protein kinase C (PKC) pathway is an additional mechanism by which hyperglycemia causes injury in complications-prone tissues. Elevated glucose levels stimulate diacylglycerol (DAG), which in turn activates PKC. Increased production of the PKC (PKC- β -isoform in particular) has been implicated in over expression of the angiogenic protein, vascular endothelial growth factor (VEGF), neural factor κ B (NF- κ B), transforming growth factor β (TGF- β) and the development of diabetic complications such as retinopathy, nephropathy, neuropathy and cardiovascular disease (Edward *et al.*, 2008).

2.5.5.3 Advanced glycation end products (AGEs):-

Advanced glycation end products formation is an additional pathogenetic mechanism of DPN. Protein glycation is a spontaneous reaction and is dependent on the degree and duration of hyperglycemia, the half-life of the protein and permeability of the tissue to free glucose (Ahmed, 2005). Increased glycation and build-up of tissue AGEs have been implicated in diabetic complications because they can alter enzymic activity, decrease ligand binding, modify protein half-life and alter immunogenicity. Glycation-derived free radicals can cause protein fragmentation and oxidation of nucleic acids and lipids (Obrosova, 2008). Interaction of AGEs with their cellular receptors has an important role in the pathogenesis of diabetic complications. Cells possess receptors for AGEs, the best studied of them is the receptor for AGE (RAGE). Receptor for AGE is a multiligand receptor and a member of the immunoglobulin superfamily of cell surface molecules and found on smooth muscle cells, macrophages, endothelial cells and astrocytes. RAGE acts as a signal transduction receptor for N ϵ -[carboxymethyl]-lysine (CML), the major AGE in vivo, although is likely to interact with other AGEs (Edward's *et al.*, 2008).

2.5.5.4 Loss of neurotrophic support:-

Growth factor deficiency may also play a role in the pathogenesis of diabetic neuropathy. Neuronal growth factors promote survival, maintenance, and regeneration of neurons subjected to the noxious effects of diabetes, the success of diabetic patients in maintaining normal nerve morphology and function may depend on expression and efficacy of these factors (Obrosova, 2008). Diabetic neuropathy is characterized by neuronal

degeneration and damage to supporting Schwann cells, perturbations in growth factors (growth factors promote the growth and survival of neurons and direct neurite outgrowth) such as nerve growth factor (NGF), insulin-like growth factor (IGF), and neurotrophin 3 (NT-3) have been suggested to be involved in the pathogenesis of diabetic neuropathy (Edward's *et al.*, 2008).

2.5.5.5 Immune Mechanisms:-

In some patients, immune mechanisms may be responsible for the clinical neuropathic syndrome, especially in those with proximal neuropathy and those with a more marked motor component to their neuropathy. Circulating antineuronal antibodies are present in diabetic serum in some patients. A cross-sectional analysis of 154 patients with diabetic neuropathy revealed that 12% had positive anti-GM1- ganglioside antibody (an antibody subtype) findings (Vinik, 1999).

2.5.5.6 Role of oxidative stress:-

Enhanced oxidative stress resulting from imbalance between production and neutralization of reactive oxygen species (ROS) is a well recognized mechanism in the pathogenesis of DPN and other diabetic complications (Obrosova, 2008). Diabetes will result in: accumulation of free lipid peroxidation product, glutathione (GSH) depletion and increase in oxidized to reduced glutathione (GSSG/GSH) ratio, and down regulation of superoxide dismutase (SOD) activity in the diabetic peripheral nerve leading to oxidative stress in diabetic peripheral nerve. Accumulation of nitrotyrosine (a footprint of peroxynitrite- induced protein nitration) has been documented in peripheral nerve, spinal cord, vasa nervorum, and dorsal

root ganglia (DRG) in both Type 1 and Type 2 diabetic rodent models indicating that diabetes creates not only oxidative, but oxidative–nitrosative stress in peripheral nervous system (PNS) (Ziegler, 2006).

2.5.5.7 C-peptide deficiency:-

C-peptide is a segment of the proinsulin molecule sliced off to form insulin. Acting through both its own receptors and modulating activity of insulin receptors, C-peptide produces multiple insulin and insulin like growth factors (IGF) effects. C-peptide insufficiency has an important role in the pathogenesis of Type 1 diabetic complications. In agreement with this implication, in patients with recently diagnosed Type 1 diabetes, C-peptide treatment was shown to correct sensory nerve conduction velocity (NCV) and vibration perception (Dobretsov *et al.*, 2007).

2.5.5.8 Microvascular Insufficiency:-

Microvascular insufficiency has been implicated as a cause of diabetic neuropathy. Studies suggest that absolute or relative ischemia exists in the nerves of diabetic persons due to altered function of the endoneurial and/or epineurial blood vessels. Studies show decreased neural blood flow, increased vascular resistance, decreased po_2 , and altered vascular permeability characteristics such as loss of the anionic charge barrier and decreased charge selectivity (Vinik, 1999).

2.5.6 Pathological background:-

Most pathological studies up to date on diabetic neuropathy have been conducted on autopsy materials or biopsied samples of sural nerve obtained from diabetic patients with established neuropathy. The main morphological

features of established neuropathy include a combination of demyelination and axonal degeneration of myelinated fibers, degeneration with regeneration of unmyelinated fibers and endoneurial microangiopathy, with nerve fiber loss in its final stage (Dobertsov *et al.*, 2007). One particular characteristic of diabetic neuropathy is the presence of endoneurial microangiopathy that parallels with nerve fiber loss, multiplicated basement membranes and swollen endothelial cells, similar to those encountered in other sites of diabetic patients, including skin, eye, kidney, and muscles. The progressive nerve fiber loss may be attributed to ongoing axonal degeneration of wallerian type as well as dying back type or secondary to severe demyelination. If regeneration fails, degenerated fibers will be lost (Yagihashi *et al.*, 2007). Demyelination occurs when there is a dysfunction of Schwann cells or damage to myelin sheath. The loss of myelin results in shunting of current across the denuded internodal axolemma. This in turn leads to attenuation of action potential; therefore, the conduction through the demyelinated nerve may be slowed or blocked. As the current density of the action potential is diminished, the time needed to achieve threshold at each node increases, hence conduction velocity is reduced (Dyck and Thomas, 2005).

2.5.7 Risk factors of diabetic peripheral neuropathy:-

The duration of diabetes and degree of metabolic control are the two major predictors of the development of neuropathy and determinant of its severity. Other factors, such as patient's age, height, and presence of proliferative retinopathy, nephropathy, and cardiovascular diseases, have also been implicated (Box 2.4). The role of excessive alcohol consumption as an

independent risk factor for the development of diabetic neuropathy has also been emphasized and the role for smoking is less clear. (Booya *et al.*, 2005).

Box 2.4 Risk factors for diabetic peripheral neuropathy (Harati, 2007)

- Older age
- Male sex
- Height
- Longer duration of diabetes
- Poor glycemic control
- Nicotine use
- Hypertension
- Hyperlipidemia
- Alcohol

2.5.7.1 Age:-

It is an important risk factor for DPN, older age diabetics are at higher risk for development of DPN (Van Acker, *et al.*, 2009). A study reported that the incidence of diabetic neuropathy increased with age and reached 44% in older diabetics (Kawano *et al.*, 2001).

2.5.7.2 Gender:-

Many studies found out a significant difference in age of onset and the severity of the signs and symptoms of diabetic neuropathy between males and females. One of such study (Brown *et al.*, 2008) determines that most patients with diabetic neuropathy are males, with the duration of their DM being approximately 10 years. Another study (Aaberg *et al.*, 2008) suggests

that, males are presented with the complication of diabetic neuropathy approximately 4 years earlier than their female counterparts.

2.5.7.3 Height:-

Taller diabetic patients seem more vulnerable to peripheral neuropathy, in keeping with the length-dependent presentation of diabetic polyneuropathy (Coppini *et al.*, 2001; Tesfaye *et al.*, 2006).

2.5.7.4 Duration of diabetes mellitus:-

Diabetes duration is also independently associated with microvascular complications. Longer duration of diabetes is a risk factor for development and progression of microvascular complications and it is nearly universal finding in previous studies (Kastenbauer *et al.*, 2003; Sandberg and wickblad, 2003; Brown *et al.*, 2004; Shiraiwa *et al.*, 2005; Costa *et al.*, 2006; Tesfaye *et al.*, 2006; Kiziltan *et al.*, 2007 and Cardoso and Salles, 2008).

2.5.7.5 Hyperglycemia:-

Hyperglycemia clearly plays a key role in the development and progression of diabetic neuropathy as well as other microvascular complications of diabetes. Diabetic neuropathy is thought to occur from both hyperglycemia induced damage to nerve cells per se and from neuronal ischemia caused by hyperglycemia-induced decreases in neurovascular Flow (Park *et al.*, 2004). Long term hyperglycemia elicits enhanced polyol pathway, increased nonenzymatic glycation of structural proteins, increased oxidative stress as well as altered protein kinase C activity and poly ADP-ribose polymerase (PARP) activation that are all interrelated for the cause and development of neuropathy. These in turn activate or suppress the

protein kinase C activity or activate mitogen activated protein kinase (MAP) activity, resulting in functional and structural derangement of peripheral nervous system. It is yet to be clear, how these mechanisms are related each other or independently operate (Dobertsov *et al.*, 2007). The Diabetes Control and Complications Trial (DCCT) demonstrated that intensive treatment in patients with Type 1 DM significantly reduced both the incidence and progression of neuropathy (Bragd *et al.*, 2008). The United Kingdom Prospective Diabetes Study showed that intensive treatment of diabetes reduced the risk of development of microvascular complications in patients with Type 2 diabetes (Ozmen, and Boyvada, 2003). A prospective follow-up study was carried out in Brazil with Type 2 diabetic outpatients. The main finding of this prospective study with up to 7 years (range 2–84 months) of follow-up is that poor metabolic control, both glycemic and lipidic, and poor hypertension control, reflected by increased left ventricular mass and hypertrophy, were the most important independent predictive factors for future development and progression of microvascular disease in Brazilian Type 2 diabetic patients (neuropathy occur in 15.5% of the patients). The study also demonstrated that mean fasting glycemia obtained during the first year of follow-up predicted future occurrence or progression of any microvascular disease and, also specifically, of retinopathy and peripheral neuropathy but not of clinical nephropathy (Cardoso and Salles, 2008). In a case-control study, hyperglycemia was the only modifiable risk factor for diabetic neuropathy. The control of blood sugar (glycemic control) reduces the incidence of neuropathy, slows its progression and improves the diabetic patient's quality of life (Booya *et al.*, 2005). Strict glycemic control can improve diabetic peripheral neuropathy in patients with Type 1 diabetes, and prevent its progression in patients with Type 2 diabetes. In addition,

there is some evidence that both long- and short-term glycemic control can improve vibratory sensation (as an indicator of status of sensory nerve) in patients with Type 1 diabetes (Fujita *et al.*, 2008). In a cross sectional study on subjects with Type 2 diabetes mellitus done by Shiraiwa *et al.*, (2005) found out that post-prandial hyperglycemia, rather than fasting glycemia or hemoglobin A1c levels, was an important predictor of the incidence of diabetic neuropathy.

2.5.7.6 Smoking:-

Smoking may serve as an independent risk factor for peripheral neuropathy. Smoking and agents contained in nicotine induce an increase in insulin resistance, an abnormal state in which an impairment of cellular insulin signaling results in an overt resistance to the physiologic effects of insulin in terms of disposal of glucose and suppression of gluconeogenesis. This state leads to a combination of hyperinsulinemia and hyperglycemia, endothelial cell dysfunction, and hypertension and microvascular complications. It is not clear whether cessation of smoking results in the improvement or slower progression of these complications, but it is prudent that patients be encouraged to do so (Harati, 2007). Booya *et al.*, (2005) found out that there was no correlation between smoking and DPN. In another study (Eliasson, 2003), in young insulin-treated diabetic subjects, it was shown that, apart from glycemic control, cigarette smoking as well as height, and female gender may be independent risk factors for progression of distal sensory neuropathy.

2.5.7.7 Hypertension:-

Is a strong predictor of development and progression of diabetic microvascular complications, either aggregated or in isolation (Schillaci *et al.*, 2000). Many studies showed that the subgroup of patients with the

highest level of both HbA1c and systolic blood pressure had a 16-fold increased risk of developing microvascular disease in comparison with the subgroup in the lowest category (Cardoso and Salles, 2008). Booya *et al.*, (2005) and Van Acker *et al.*, (2009) found out that there was no correlation between hypertension and DPN.

2.5.7.8 Lipid profile abnormalities:-

The relationships between serum lipid levels and development of diabetic microvascular disease are controversial. Many studies show a positive association between lipid levels and microvascular complications, but results are inconsistent with regard to which lipid fraction is most predictive (Leiter, 2005). Some studies found that there is a protective effect of serum HDL cholesterol level on peripheral neuropathy development (probably because it is protective against peripheral vascular disease which determines progression of peripheral neuropathy) (Booya *et al.*, (2005); Agrawal *et al.*, (2006); Tesfaye *et al.*, 2006 and Cardoso and Salles, 2008) found out that there was no correlation between lipid profile abnormalities and DPN. Van Acker *et al.*, (2009) found that low HDL cholesterol and raised triglyceride levels independently associated with DPN while high total cholesterol were not associated with DPN.

2.5.7.9. Vitamin E (α -tocopherol):-

Vitamin E is a fat-soluble compound that exists in eight isoforms with varying biological activity. α -Tocopherol is the most active isoform and is the most common dietary supplement of vitamin E. This compound has been broadly tested for its ability to prevent chronic diseases involving oxidative stress including cancer and diabetes complications. In addition to potent antioxidant action, vitamin E can promote immune function, DNA repair,

and metabolism. Chronic vitamin E administration improves the ratio of cardiac sympathetic to parasympathetic tone in patients with Type 2 diabetes when given 600 mg/day for 4 months (Edward's *et al.*, 2008).

In another study, the levels of Vitamin E were significantly lower in patients with diabetic foot ulcers as compared to the uncomplicated diabetes group and the normal controls. The highest levels of oxidative stress in diabetics have been shown to decrease following supplementation with antioxidants like Vitamin E (Singh *et al.*, 2008). Sandhu *et al.*, (1999) reported lower levels of antioxidants like Vitamin E and β -carotene in DPN patients than control group.

2.5.7.10 Serum Selenium:-

Selenium is a trace element whose essentiality in mammals was discovered in 1957. Selenium has a large number of biological functions in the human being (Driskell *et al.*, 2006). The most important and known action is its antioxidant effect. A deficiency in selenium would originate in impairment in the glutathione peroxidase (GSH-Px) activity, whose main action is to catalyze the reduction of the organic and inorganic hydroperoxides produced during the oxidative stress of the phospholipids of the cell membrane. Deficiency of selenium may result in enhancement of oxidative stress and may cause or aggravate chronic diseases (Tinggi, 2003). The organic antioxidant systems together with the catalase, superoxide dismutase, vitamin E, vitamin C, carotenoids, *etc.*, whose principal function is to eliminate the free radicals derived from oxygen such as hydrogen peroxide, organic hydroperoxides, superoxides and hydroxyl radicals. The indicated antioxidant activity of the selenium seems to be exerted synergistically with vitamin E (Pedrero and Madrid 2009). Selenium has also been found to have

insulin-mimetic activities in vitro and in vivo and has been shown to stimulate glucose uptake in adipocytes in vitro, restore normoglycaemia in streptozotocin-diabetic rats and regulate vital metabolic processes such as glycolysis and gluconeogenesis. The mechanisms by which selenium exerts these effects are still relatively unclear, however evidence indicates that selenium may activate key molecules in the insulin signaling cascade (Campbel *et al.*, 2008).

2.5.8 Assessment of diabetic peripheral neuropathy:-

It includes clinical (history and examination), biochemical and electrophysiological assessment.

2.5.8.1 Clinical assessment:-

A- Clinical history:-

- 1- History of DM regarding duration, type of treatment, degree of control, onset of disease to determine type of DM.
- 2- Presence of any symptom of neuropathy, its severity, site and duration.
- 3- History of any other disease or drug intake that could cause or aggravate neuropathy (Karsidag *et al.*, 2005).

B-Physical examination:-

Physical examination includes general examination to identify any sign of disease that causes neuropathy other than diabetes like; anemia (vitamin-B12 deficiency), thyroid enlargement, petechial hemorrhage indicating vasculitis and others.

Full neurological examination starting by inspection looking for ulcer, deformity, amputation, fissuring of dorsum of foot, loss of hair and coldness indicative of autonomic neuropathy or peripheral vascular disease. After that

sensory and motor examination should be done (Costa *et al.*, 2006). Complete sensory examination that include; pinprick test, fine touch, vibration perception by 128 tuning fork, proprioception and thermal sensation. All these tests should be done to all the 4 limbs and trunk with comparison between proximal and distal sensation. Also the testing should be focused on big the toe because early signs of DPN begin distally. Complete motor examination should be done including muscle tone, power and reflexes with attention to the ankle reflex which is lost early in DPN (Unger and Cole, 2007).

2.5.8.2 Glycosylated Hemoglobin (HbA1c):-

Hemoglobin A1c (HbA1c) is a minor component of hemoglobin A to which glucose is bound. It is referred to as glycosylated, or glycated hemoglobin. HbA1c is formed within the red blood cells at a rate directly proportional to the blood glucose level. Thus, the average amount of HbA1c changes in a dynamic way and reflects the mean blood glucose concentration over the previous six to eight weeks (Nathan *et al.*, 1994). Clinically the measurement of HbA1c is used for the evaluation of the glycaemic control over the last 8 weeks (Goldstein, 1999).

2.5.8.3 Biochemical Assessment:-

A-Fasting blood sugar measurement:-

Fasting blood sugar provides accurate assessment of diabetic control. Fasting samples should be obtained after a period of 8-12 hours after the last food intake.

B- Lipid profile (total cholesterol, HDL, LDL, VLDL and triglyceride)

The most important plasma lipids are triglyceride, free and esterified cholesterol, phospholipids, and free fatty acids. The water insoluble lipids

are made soluble in plasma by their association with certain proteins, the apolipoproteins (Havel and Goldstein 1997).

Lipoproteins fall into four relatively discrete classes i.e, chylomicrons, very low density lipoprotein-triglycerides (VLDL-triglycerides), low density lipoprotein-cholesterol (LDL-cholesterol), and high density lipoprotein (HDL) (Fried *et al.*, 2001).

Hypertriglyceridaemia, hypercholesterolaemia, elevated fatty acid concentration and increased level of low-density lipoprotein (LDL) have been reported in diabetic patients. Among these abnormalities, hypertriglyceridaemia is the most common, in which a defective clearance of triglycerides is implicated (Davidson's, 2006).

C- Vitamin E level:- described in page 24

D- Selenium level:- described in page 25.

(Maple-Brown *et al.*, 2008).

2.5.8.4 Electrophysiological assessment:-

Nerve conduction studies are the most informative portion of the electrodiagnostic evaluation for peripheral neuropathy. The sensitivity of nerve conduction studies in identification of neuropathy is extremely high; being about 90% in most focal and diffuse neuropathies if the proper tests are selected for a given disorder and the testing is technically satisfactory (Yasuda *et al.*, 2007). Their usefulness can be enumerated in a variety of ways:-

- 1- Detecting and documenting peripheral abnormalities, and its precise localization (neuronal cell body, roots, plexus, or peripheral nerve).
- 2- Confirming the anatomic distribution of a neuropathy, that is, mononeuropathy, multiple mononeuropathy (mononeuritis multiplex), or symmetric polyneuropathy.

- 3- Most important, electrodiagnostic studies can determine the form of the underlying pathological process affects primarily the axons, their myelin sheaths or both and its distribution (focal, multifocal or generalized).
- 4- Following the course of a polyneuropathy, they may aid in the assessment, prognosis and the effect of treatments.
- 5- Stating the severity of the disease process (Greenberg, 2004).

Nerve conduction study comprises two parts, motor and sensory nerve conduction studies.

2.5.8.4.1 Motor nerve conduction study:-

The electrical response obtained by stimulation of motor nerve is called compound muscle action potential (CMAP), which represents summated electrical activity of the muscle fibers that are in the region of recording electrode and that are innervated by the nerve. The following parameters are recorded for all motor nerves and then compared with the control subjects: latency, amplitude, configuration of the wave and conduction velocity (Karsidag *et al.*, 2005). Latency or distal motor latency (DML) is the time in millisecond from the application of the stimulus to the initial deflection from the baseline, either positive or negative, and it is the time required for the action potential in the fastest conducting fibers to reach the nerve terminals and activate the muscle fibers. In nerve diseases like DPN, DML will prolong and this prolongation is comparable to the degree of nerve damage (Kimura, 2001). Amplitude is the height in milivolt from the baseline to the peak of the negative phase and it is proportional to the number of muscle fibers activated and provides an estimate of the amount of functioning nerve and muscle. In DPN the amplitude is reduced below normal indicating axonal neuropathy. Conduction velocity is the speed of conduction of nerve

fibers and measured by meter/second, nerve conduction velocity is also reduced in DPN (Uluc *et al.*, 2008).

2.5.8.4.2 Sensory nerve conduction studies:-

In most cases it is performed at the beginning of standard electrodiagnostic testing. Sensory studies are often the first approach to determine the presence of a neuropathy (David, 2001). The sensory nerve action potential (SNAP) represents the sum of the individual nerve fiber action potentials recorded from a sensory nerve or distal sensory nerve endings and reflects the integrity of the dorsal root ganglion cells and their peripheral axons. Sensory nerve conduction study is done for median, ulnar and sural nerves. Latency, amplitude and conduction velocity are measured for sensory conduction studies as they are for motor conduction study (Aminoff, 2005). The parameters of sensory nerve conduction study are shown in Figure 2.2

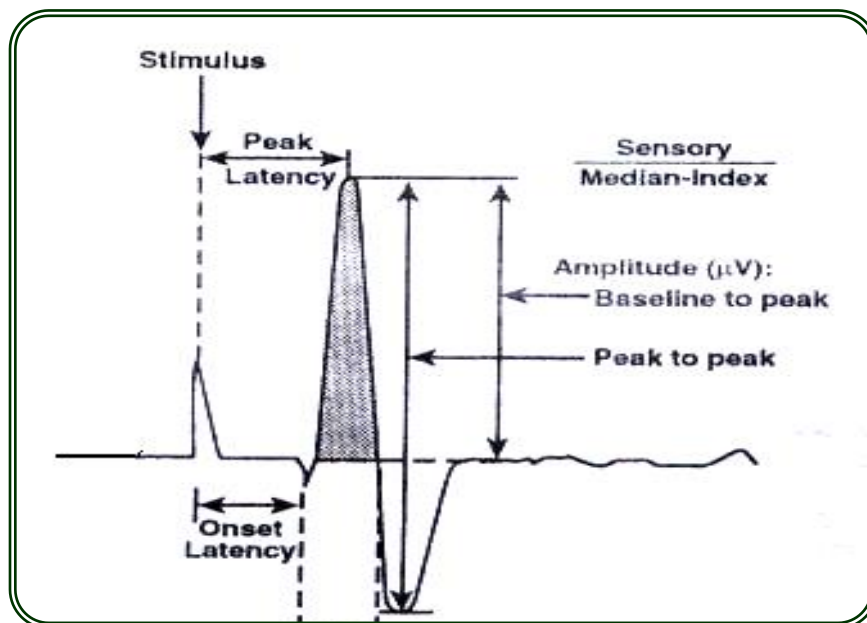


Figure 2.2: Measurement of sensory nerve action potential parameters (Aminoff, 2005).

2.5.8.4.3 The F-wave:-

The F-wave is a late compound muscle action potential resulting from the backfiring of antidromically activated motor neurons, which can be recorded with a supramaximal electrical stimulus from almost every skeletal muscle. It was so named because it was initially recorded in the foot muscles. Electrical stimulation of motor fibers results in an impulse that travels both orthodromically toward the muscle and antidromacally toward the spinal cord. The short latency direct response from orthodromic conduction is the M-wave, and the late response occurring after the M-wave is termed the F-wave (Kimura, 2001). Measurements of F-wave latencies supplement routine nerve conduction studies since they also evaluate the proximal conduction in motor fibers. They are of value in disorders involving the nerve roots, plexuses, and the proximal segments of peripheral nerves (Ali, 2007).

2.5.8.4.4 Electrophysiological changes in diabetic peripheral neuropathy

Nerve conduction study is sensitive and specific diagnostic technique for DPN (sensitivity 20.8%, specificity 100%, positive predictive value 100%, and negative predictive value 12.3%) (Costa *et al.*, 2006). Nerve conduction studies have long been considered gold standard for the diagnosis of all neuropathies, and are, most simply considered, an extension of the clinical neurological examination. Nerve conduction studies assess peripheral motor and sensory function by recording the evoked response to stimulation of peripheral nerve.

Nerve conduction study abnormalities are detectable in 29–70% of Type 1 patients at initial diagnosis and improve after institution of therapy for

hyperglycemia (Mrad *et al.*, 2000), and are present at diagnosis in 45–60% of subjects with Type 2 diabetes (Akbar *et al.*, 2000). Its value in the diagnosis of DPN is the following:-

- 1- Nerve conduction study has helped to define the pathophysiological features of DPN.
- 2- Nerve potential amplitudes reflect the degree of nerve fiber loss which represents the pathological hallmark of DPN (Perkins and Brill, 2001).
- 3- The prevalence of abnormal NCS increases with duration of diabetes and disease severity correlates with glycemic control.
- 4- Side-to-side symmetrical nature of DPN is reflected by NCS (Hyllienmark *et al.*, 2001).

The most distal sensory nerves (sural) typically provide the first evidence of DPN. As DPN progresses, the NCS show loss of distal nerve sensory and motor amplitudes, and then changes in more proximal and upper limb nerves (Uluc, *et al.*, 2008). While DPN is an insidiously progressive disorder, the rate of progression may not be linear and generalized for all nerve fibers. The small changes in conduction velocity are readily demonstrable, but amplitude changes are more difficult to show due to higher variability of this measure, although in the long term, the amplitudes of the responses decline inexorably (Wilson *et al.*, 2005).

Some investigators suggested that sural nerve dysfunction is the most common indicator of peripheral nerve dysfunction, and it is the first to be affected, and correlates most closely with the neuropathological findings. A study found out that the peroneal motor nerve had the highest abnormality, followed by the sural nerve, median sensory and median motor nerves (Turgut *et al.*, 2004). In another study, the most affected nerves were the sural sensory nerve, peroneal motor nerve, posterior tibial motor nerve,

median motor nerve, ulnar motor nerve, median sensory nerve, and ulnar sensory nerve. So it is suggested that the most useful and practical nerves for the electrophysiological study in diabetic patients are the motor and sensory nerves of lower extremity (Karsidag *et al.*, 2005). Moreover, to increase the sensitivity of NCS for diagnosing DPN, the amplitude ratio between sural and radial sensory responses has been used (Kikawa *et al.*, 2005). Another measure used to increase the sensitivity of NCS for the diagnosis of DPN is the measurement of refractory period which has been shown to be a useful measure of subtle nerve dysfunction. Following an action potential, large myelinated axons are absolutely refractory for 0.5–1.0 ms, during which time they cannot generate another action potential no matter how strong the stimulus. The axons are then relatively refractory for another 3–4 ms, during which time a stronger than normal stimulus is required to generate an action potential (Burke *et al.*, 2001; Misawa *et al.*, 2004). Kuwabara, (2003) analyzes single sensory units in the median nerves in Type 1 or Type 2 diabetics. Conduction velocities did not differ between diabetic and normal subjects, but absolute refractory periods of diabetic axons ranged from 0.9 to 2.2 ms (mean, 1.3 ms), and were significantly shorter than the normal axons range of 0.9–3.5 ms (mean, 1.8 ms). Since the dorsal sural nerve of the foot is the most distal and sensory nerve, they may be affected in the early stage of polyneuropathy; in addition it has been reported that the dorsal sural nerve recording is reliable in the diagnosis of early peripheral neuropathy (Killian and Foreman, 2001). At early stages of the DPN, the disturbances in sensory nerve conduction studies will predominate with normal motor nerve study parameters, which have been taken as evidence for (sensory) DPN (Uluc *et al.*, 2008). In a study done by Meijer *et al.*, (2008) invasive muscle fiber conduction velocity (MFCV) testing was shown capable to

detect muscle fiber conduction slowing due to DPN acute axonal loss resulted in a progressive slowing of MFCV, mainly resulting from muscle fiber atrophy. This study shows that MFCV measurements offer a sensitive method in detecting early motor dysfunction in DM.

2.5.9 Diagnosis of diabetic peripheral neuropathy

Diabetic peripheral neuropathy can be diagnosed by variety of tests that provide definite diagnosis:-

- 1- Taking full history and neurological examination is the corner stone in the diagnosis of DPN, providing that it is done by experts.
- 2- Standard electrophysiologic methods have also been used extensively to diagnose and follow the progression of DPN (Gin *et al.*, 2006). However, because there is little demyelination in the early stages, changes in maximal conduction velocity are gradual (0.5– 0.7 m/s per year) and, thus, the onset of DPN-induced deficits may be difficult to distinguish. Furthermore, whole nerve electrophysiology exclusively measures function in large-diameter neurons, and the spectrum of nerve fibers involved in DPN includes both the large-diameter, heavily myelinated axons ($A\alpha$ and $A\beta$) that convey vibratory and tactile information, and small-diameter, thinly myelinated ($A\gamma$) or unmyelinated (C) fibers that convey information about pain and temperature. Nerve fiber types may not be uniformly affected in DPN; thus, electrophysiology, particularly conduction velocity alone, may provide a poor measure of early dysfunction in some patients. Although response amplitudes can be correlated with fiber density in a population, the onset of change in this measure may not be apparent in individual patients because of the considerable variability in amplitude measure (Unger and Cole, 2007).

3-Nerve biopsy is not recommended for routinely in the evaluation of patients with DPN.

4-Several highly sensitive methods have recently been developed for evaluating changes in peripheral nerve structure and function in patients with diabetes like Skin-Punch biopsy and immunohistochemical staining for peripheral nerve axons and peripheral nerve imaging using MRI (Arezzo, 1999).

2.5.10 Treatment of diabetic peripheral neuropathy

A- Glycemic Control

Retrospective and prospective studies suggest a relation between hyperglycemia and the development and severity of diabetic neuropathy. So attempts at preventing or slowing progression of neuropathy focus on controlling hyperglycemia (Dyck and Thomas, 2005).

B- Aldose Reductase Inhibitors

Aldose reductase inhibitors (ARIs) reduce the flux of glucose through the polyol pathway, inhibiting tissue accumulation of sorbitol and fructose and preventing reduction of redox potentials. There are many known aldose reductases like alrestatin, tolrestat, zenrestat and others (Edward's *et al.*, 2008).

C- Advanced Glycosylation End Products

Advanced glycosylation end product (AGE) inhibition may be of some value in the treatment of diabetic neuropathy. Studies in rats with streptozocin-induced diabetes using the AGE inhibitor aminoguanidine showed

improvement in nerve conduction velocity. Controlled clinical trials are needed to determine its efficacy in humans (Vinik, 1999).

D- Antioxidants

Given the known mechanisms leading to diabetic neuropathy, a logical therapeutic approach is to prevent oxidative stress by increasing antioxidant defense. This makes oral antioxidants an attractive strategy for both prevention and treatment of diabetic neuropathy. Many clinical trials of antioxidant defense therapies have been completed, mostly using a high dose of a single antioxidant compound. The results of these trials have been largely negative, despite the strong rationale for this approach. There are many antioxidants known like, Vitamin E, α -lipoic acid, resveratrol and others (Kumar *et al.*, 2007).

E- Controlling pain of diabetic peripheral neuropathy

Pain is a major troublesome symptom of DPN. There are many drugs used to control this pain like, Gabapentin, tricyclic antidepressant, anticonvulsant and others (Wamboldt *et al.*, 2006)

CHAPTER THREE

Materials and Methods

3.1 Materials:-

3.1.1 Subjects:-

The study was conducted in diabetes center in Marjan teaching hospital in AL-Hilla City. The total number of subjects involved in the study was 225 (105 patients & 120 controls). The study group consisted of 87 male and 138 female, 50 had Type 1 DM and 115 had Type 2 DM. The age distribution of study group ranged from 16-72 years. The patients with diabetic polyneuropathy included in the study were classified according to the type of DM into:- patients with Type 1 DM (15 male & 20 female, total 35) & patients with Type 2 DM (22 male & 48 female, total 70). The patients were recorded in the diabetes center and had monthly visit. They were asked if they had any symptoms of peripheral nerve dysfunction like parasthesia, numbness, pain, weakness or others. Patients who experienced any of these symptoms were included in the study. The control group had the same sex and age group distribution to the patients. The control group was subdivided into diabetic patients without neuropathy (60 patients) and normal healthy control (60 persons). Diabetic patients without neuropathy were classified into:- patients with Type 1 DM (5 male & 10 female, total 15) & patients with Type 2 DM (12 male & 33 female, total 45). The normal healthy control group were classified according to the age into those with age group between (16-40 years) to be considered as the control of Type 1 DM (13 males and 9 females, total 22) & those with age group between (50-65 years) that considered as control of Type 2 DM (20 males and 18 females, total 38). The distribution of the subjects according the subgroup is

shown in the following table (table 3.1). The control group underwent the same tests and exams as those for patients.

A specialist doctor assessed all subjects included in the study and differentiated between patients and control.

Table 3.1 Distribution of study group.

Variable	Diabetic peripheral neuropathy patients		Diabetic without neuropathy		Healthy controls		Total
	Type 1	Type 2	Type 1	Type 2	Type1	Type2	
Age (year)	16-34	45-72	18-26	42-52	16-40	50-65	
males	15	22	5	12	13	20	87
Females	20	48	10	33	9	18	138
Total	35	70	15	45	22	38	225

3.1.2 Chemicals used in the study:- shown in the lower table (3.2):-

Table 3.2 the chemicals used in the study

Chemical material	Company	Country
Glucose kit	Human	Germany
Total cholesterol & TG kit	Human	Germany
HDL kit	Biolabo	France
HbA1C kit	Stanbio lab.	USA

3.2.2 Instruments used in this study:- shown in the following table (3.3)

Table 3.3 the Instruments used in this study

Instrumental	Supplied Company	Country
Spectrophotometer	Apel	Japan
Centrifuge	Sigma	Germany
Micropipette	Slamed	Germany
Distillator	Bibby science	England
Centrifuge tube	Mes(1 gm) Fisonsm	England
Plane tube	Afma-Dispo-	Jordan
EDTA tube	Afma-dispo	Jordan
Disposable syringe	Witeg	Malaysia
Tuning fork	Rossmax	USA
Nerve conduction study machine	Micromed	Japan
Atomic absorption machine	Shimadzu AA-670	Germany

3.2.3 Assessment:-

All the patients and control groups were assessed clinically, biochemically and electrophysiologically as following:

3.2.3.1 Clinical assessment:-

3.2.3.1.1 History:-

Full history were taken from each patient regarding personal data like name, age, sex,..... *etc*, history of presenting illness (polyneuropathy) focusing on symptoms like parasthesia, numbness, pain, exaggerated painfull sensation to normal painless stimuli (allodynia), loss of thermal sensation, weakness, shoe slipping, history of foot ulcer or injury, and others distribution of these symptoms, duration of the symptoms, duration of DM, type of treatment of DM, history of other disease, and history of drug intake (Cardoso and Salles, 2008).

3.2.3.1.2 Examination:-

It was in the form of general and systemic examination including full neurological examination. The neurological examination consisted of:-

- 1- Inspection looking for atrophy, abnormal gait, deformity, ulcer, fasciculation and others.
- 2- Examination of motor system (muscle tone, power, reflexes and coordination).
- 3- Examination of sensory system (pinprick, thermal, light touch, proprioception and vibration sensation). The neurological examination focused on lower limbs, especially medial malleollus and big toe (Nather *et al.*, 2008). The same trained examiner tested all participants.

3.2.3.1.3 Blood Collection:-

Venous blood samples were aspirated at about 9 a.m. from antecubital fossa. From each person, ten ml of blood aspirated, 4 milliliter of blood (anticoagulated with EDTA) used for glycosylated hemoglobin

measurement, and other part placed in centrifuge for 10 minutes after waiting for 45 minutes to separate serum from whole blood. Serum samples stored in refrigerator (-20c°). Serum samples were used for measurement of blood sugar, total serum cholesterol, HDL - cholesterol and triglyceride, selenium & vitamin E measurement (Lewis *et al.*, 2006).

3.2.3.2 Glycosylated hemoglobin measurement:-

Whole blood preparation was mixed with a weakly binding cation-exchange resin; the non-glycosylated hemoglobin was bound to the resin, leaving HbA1c. The percent of HbA1c was determined by measuring the absorbance values at 415nm of the HbA1c fraction to the total Hb fraction, according to the procedure explained by the company (Stanbio lab., USA)(Frank, 1988).

Reagents composition:

Reagent	Initial concentration of solution
Ion exchange resin	8mg/dl
Lysing reagent	10mmol/L

Procedure

a-Hemolysate preparation

1-A quantity of 0.5ml of lysing reagent was pipette into tubes labeled standard, unknown and control.

2- A quantity of 0.1 ml of well mixed blood sample was pipette into the tubes and mixed. The tubes was allowed for 5 minutes at room temperature to complete hemolysis.

b-glycohemoglobin separation and assay:

1- Resin tubes were labeled as standard, unknown and control.

2- A quantity of 0.1ml hemolysate was pipette into the appropriately labeled resin tubes.

3-the tubes was mixed manually for 5 minutes.

4-each supernatant was poured directly into separate cuvettes for absorbance at 415 nm.

c-Total hemoglobin assay

1-Deionized water (0.5 ml) was pipette into tubes labeled as standard, unknown and control.

2- a hemolysate (0.02 ml) was poured into appropriately labeled tubes, mixed well and transferred into cuvette for absorbance reading at 415 nm.

Calculation

The ratio (R) for each tube was measured by equation:-

$$R = \frac{\text{absorbance of glycohemoglobin}}{\text{absorbance of total hemoglobin}}$$

$$\text{Glycohemoglobin\%} = \frac{R(\text{unknown})}{R(\text{standard})} \times \text{concentration of glycohemoglobin standard}$$

3.2.3.3 Biochemical Evaluation:-

3.2.3.3.1 Serum Glucose measurement:-

Glucose is oxidized by glucose-oxidase to gluconate and hydrogen peroxide according to the following equation.



The absorbance of standard & sample are measured against reagent blank at 546nm according to the procedure recommended by the company (Human, Germany) (Burits and Ashwood, 1999).

3.2.3.3.2 Determination of Serum Cholesterol:-

Cholesterol is determined according to the following reaction:-
cholesterol ester $\xrightarrow{\text{cholesterol esterase}}$ cholesterol + fatty acids

Cholesterol $\xrightarrow{\text{cholesterol oxidase}}$ cholest-4-en-3-one + H₂O₂

2H₂O₂ + phenol + 4-amino $\xrightarrow{\text{antipyrine Peroxidase}}$ quinoneimine + 4H₂O

The quantity of red dye quinoneimine formed was proportional to the cholesterol concentration, and was measured by spectrophotometer at 505nm wave length.

Reagents composition:

Reagent	Initial concentration of solution
4-aminoantipyrine	0.30 mmol/l
Phenol	6 mmol/l
Peroxidase	>0.5 u/ml
Cholesterol estrase	>0.15 u/ml
Cholesterol oxidase	>0.1 u/ml
Pipes buffer	80 mmol/l
standard	5.17 mmol/l (200mg/l)

The protocol assay is carried according to the followings:

Contents	Reagent blank (μl)	Standard (μl)	Sample (μl)
Distilled water	10	–	–
Standard	–	10	–
Sample	–	–	10
Reagent	1000	1000	1000

The mixture was thoroughly mixed and incubated for 10 minutes at 20-25°C.

Calculations:

The concentration of cholesterol is calculated from the following equation:

$$\text{Concentration of cholesterol in the sample} = \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times \text{concentration of standard}$$

(Trinder, 1969; Burits and Ashwood, 1999).

3.2.3.3.3 Determination of Serum HDL-Cholesterol:-

The chylomicrons and Lipoproteins of very low density (VLDL) and low density (LDL) contained in the sample were precipitated by the addition of phosphotungstic acid in the presence of magnesium ions. The supernatant obtained after centrifugation contained high density lipoproteins (HDL).

Reagents:

Contents	Initial concentration of solution
Phosphotungstic acid	0.55 mmol/l
Magnesium chloride	25 mmol/l

The protocol assay was carried out according to the followings:

Contents	Reagent blank (μl)	Standard (μl)	Sample (μl)
Distilled water	100	—	—
Supernatant	—	—	100
Standard	—	100	—
Reagent	1000	1000	1000

The supernatant was mixed with total cholesterol reagent & left stand for 5 minutes at 37 C° and then the absorbance was recorded at 500nm against reagent blank.

Calculations:

HDL cholesterol is determined from following equation:

$$\text{The concentration of HDL in the supernatant} = \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times \text{concentration of standard}$$

(According to the procedure recommended by HDL kit from Biolabo Company, France) (Teitz, 2006).

3.2.3.3.4 Determination of Serum LDL-Cholesterol:-

The LDL concentration is calculated as follows:

$LDL = \text{Total Cholesterol} - (HDL + TG/5)$ (Andreoli *et al.*, 2004).

3.2.3.3.5 Determination of Serum Triglyceride:-

The triglycerides were determined after enzymatic hydrolysis with lipoprotein lipase. The indicator is quinoneimine.

$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipase}} \text{glycerol} + \text{fatty acids}.$

$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{glycerol -3- phosphate} + \text{ADP}$

$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{dihydroxyacetone phosphate} + H_2O_2$

$2H_2O_2 + 4\text{-aminophenazone} + 4\text{-chlorophenol} \xrightarrow{\text{POD}} \text{quinoneimine} + \text{HCL} + 4H_2O$

Reagents:

Contents	Concentration in the test
1- Buffer	
Pipes buffer	40 mmol/l
4-chlorophenol	5.5 mmol/l
Magnesium ion	17.5 mmol/l
2- Enzyme reagent	
4-amino phenazone	0.5 mmol/l
ATP	1.0 mmol/l
Lipase	$\geq \geq 150$ u/ml
Glycerol kinase	$\geq \geq 0.4$ u/ml
Glycerol-3-phosphat oxidase	$\geq \geq 1.5$ u/ml
Peroxidase	$\geq \geq 0.5$ u/ml
Standard	2.29 mmol/l

The procedure is achieved through the followings:

Contents	Reagent blank (μl)	Standard (μl)	Sample (μl)
Sample	–	–	10
Standard	–	10	–
Reagent	1000	1000	1000

The mixtures were carefully mixed and incubated for 10 minutes at 20 to 25°C. The absorbance of the sample (A sample) and the standard (A standard) were measured against the reagent blank within 60 minutes.

Calculations:

The concentration of triglycerides was calculated from the following equation:

$$\text{Triglyceride concentration (mmol/l)} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times \text{concentration of standard}$$

(Human Company, Germany) (Trinder, 1969; Burits & Ashwood, 1999).

3.2.3.3.6 determination of serum VLDL

Serum VLDL values were obtained by dividing the value of triglyceride on number five (Andreoli *et al.*, 2004).

3.2.3.3.7 Determination of Vitamin E

Preparation of reagents:-

1. α -α dipyridyle solution:

A quantity of (0.12) gm of α -α dipyridyle was dissolved in a final volume of 100 ml absolute ethanol.

2. Ferric chloride (FeCl_3): 0.4 gm of ferric chloride (FeCl_3) was dissolved in a final volume of 100 ml absolute ethanol.

Procedure:-

Vitamin -E was determined by using a Hashim & Schuttringe procedure (Hashim & Schuttringe,1966) which is summarized as follows: -

One tube is prepared as follows:

Reagents	Sample μL
Serum	500
Absolute ethanol	500
Heptane	1000

Then the tube is mixed in vortex mixture intermittently for 10 minutes, and centrifuged for 5 minutes at 1000 rpm then the outer most heptane layer (Supernatant) pipette into test tube (5 ml) and then two set of tubes are prepared as follows:

Reagents	Sample μL	Blank μL
Supernatant	800	—
Heptane	—	800
- α - α dipyridyle	600	600

Tubes are mixed and the absorbency of standards and sample was read by spectrophotometer at absorbency 460 nm.

Then the contents of each tube returned to its tube then ferric chloride (FeCl_3) 0.2 ml was added to each tube then after 45 minutes, the absorbency of standards and sample was read by spectrophotometer at absorbency 510 nm.

Calculation of Vitamin E level:

The concentration of Vitamin E level is obtained by the following equation:

$$\text{vitamin E} = \frac{(\text{Log}T_{510} - 1.63) - (0.036 \times \text{Log}T_{460})}{0.46082 \times 10^{-4}}$$

3.2.3.3.8 Serum selenium measurement:-

Serum selenium measurement was done using atomic absorption spectroscopy method in Ministry of Science and Technology. Samples are pre-treated consists only of dilution (2X) directly into auto sampler cup. Appropriate standards are prepared in 0.1 M nitric acid. The instrumental settings are shown in the following table.

Table1: Instrument setting for the determination of Selenium in serum.

Wave length of selenium 196.0 nm

Slit width 0.3 nm

Injection volume 20 μl

Electrical current 12 mA

Settings of the atomic absorption machine after injecting the sample into the auto sampler cup.

	Temp.	Time (Sec)
Drying	150	30
Atomizing	400	20
Ashing	2000	4
Cleaning	2200	4

3.2.3.4 Electrophysiological testing:-

The electrophysiological test was done in the electrophysiological department in Marjan teaching hospital. Each patient had at least four motor nerves tested (median, ulnar, tibial and peroneal), and three sensory nerves (median, ulnar and sural nerves). Limb temperatures were maintained above 33C° in the legs and 34C° in the arms, and the skin was prepared when necessary using abrasive skin cleanser and isopropyl alcohol. The nerve conduction studies were performed using a Micromed machine (Japan). Maximal responses were obtained using electrical stimuli. Distal latency, conduction velocity and waveform amplitude, duration and shape were measured and recorded for each nerve at each stimulus site (Kimura, 2001).

3.2.3.4.1 Procedure of nerve conduction study:-

The electromyography (EMG) machine consisted of the following parts:

- a. Recording machine.
- b. Electrodes.

The electrodes were stimulating electrodes that give electrical signal of controlled severity and recording electrodes were placed in fixed sites for each nerve to record the resulting nerve signal. The stimulation and recording of nerves occur through the skin (Kimura, 2001).

3.2.3.4.1.1 Sensory nerve conduction studies:

The stimulus intensity was 20-30% above the current necessary to evoke a maximal sensory nerve action potential. The supramaximal stimulating current was kept below threshold for motor fibers especially in the mixed nerves, since sensory fibers generally, had lower threshold of stimulation than that of motor fibers (Aminoff, 2005).

The sites of the electrodes for sensory nerve conduction studies for the tested nerves were as the follows:

3.2.3.4.1.1.1 Median Nerve:

Stimulation: Stimulation was applied with electrode at the plantar aspect of forearm near the wrist joint between the tendons of palmaris longus and flexor carpi radials

Recording: the recording electrode was placed over the median nerve at the index finger (Imada, *et al.*, 2007).

3.2.3.4.1.1.2 Ulnar Nerve:

Stimulation: stimulation was applied with surface electrodes at the wrist, just radial to the flexor carpi ulnaris.

Recording: the recording electrode was placed over the ulnar nerve around the fifth digit (Aminoff, 2005).

3.2.3.4.1.1.3 Sural Nerve:

Stimulation: stimulation electrodes was placed lateral to the mid-line of the calf 12cm proximal to the recording electrode.

Recording: the recording needle was inserted posterior and below the lateral malleolus of the fibula (uluc, *et al.*, 2008).

3.2.3.4.1.2 Motor Nerve Conduction Studies:

For motor nerve conduction studies, the nerve was stimulated at two points along its course, by applying stimuli at distal and proximal sites of the nerve and recording from the muscle innervated by that nerve. The stimulus

intensity must be high enough to activate all nerve fibers during stimulation (Kimura, 2001).

The sites of the electrodes for motor nerve conduction studies of the tested nerves were as follows:

3.2.3.4.1.2.1 median nerve:

Stimulation: was performed at two sites **a.** distally: at the wrist, 8 cm proximal to the recording electrode, over the skin between palmaris longus and flexor carpi radialis tendons.

b. Proximally: at the elbow, in the median aspect of the antecubital fossa, just lateral to the brachial artery.

Recording: surface electrodes were placed over the prominence of abductor pollicis brevis, between the metacarpophalangeal joint of the thumb and the midpoint of the distal wrist crease (Ali, 2007).

3.2.3.4.1.2.2 ulnar nerve:

Stimulation: was performed at two sites **a.** distally: at the wrist, 8 cm proximal to the recording electrode, and just over the flexor carpi ulnaris tendon.

b. Proximally: at the elbow just distal to the ulnar groove.

Recording: the surface electrode was placed at the abductor digiti minimi, on a point mid way between the distal wrist creases and the crease at the base of the fifth digit (Kimura, 2001).

3.2.3.4.1.2.3 Common Peroneal Nerve:

Stimulation: was performed at two sites **a.** distally: at the ankle, 8 cm proximal to the recording electrode, proximal to the lateral malleolus.

b. Proximally: at the knee, just below the head of fibula (capitulum fibulae).

Recording: The surface electrode was placed over the extensor digitorum brevis muscle (Aminoff, 2005).

3.2.3.4.1.2.4 Posterior tibial nerve:-

Stimulation: was performed at stimulation at two sites: **a-** distally, behind the medial malleolus, **b-**proximally, at the posterior surface of knee.

Recording: surface electrode was placed in front of the medial malleolus at the convexity of the foot (Aminoff, 2005).

3.3 Statistical analysis:

The arithmetic mean and standard error of distribution of each set of the data were calculated, demographic and electrophysiologic data were compared between patients and control group using a *t*-test. A P- level of 0.05 or less was considered significant (Daniel, 1999).

CHAPTER FOUR

Results

4.1 Age distribution:-

The age distribution (in years) of patients with Type 1 DM who were presented with diabetic peripheral neuropathy was 27 ± 7 years for males and 23 ± 6 years for females, for diabetics without neuropathy was 24 ± 2 years for males and 20 ± 6 years for females. The age distribution of patients with Type 2 DM who were presented with diabetic peripheral neuropathy was 54.6 ± 2.9 years for males and 53.1 ± 1.8 years for females, for diabetics without neuropathy was 49.5 ± 7 years for males and 50 ± 4 for females.

The age distribution in years of normal healthy controls for Type 1 diabetics was 34 ± 8 years for males and 30 ± 7 years for females and for healthy controls of Type 2 diabetics were 58 ± 4 years for males and 55 ± 6 years for females. There are statistically significant differences regarding age between DPN patients & diabetics without neuropathy. These values are shown in Table 4.1 (a and b).

Table 4.1a- The age distribution in the study group for Type 1 diabetes mellitus according to the sex.

Variable		Age(years)	
Type 1 diabetics	Diabetic peripheral neuropathy patients	Male 27 ± 7	Female 23 ± 6
	Diabetics without neuropathy	$24 \pm 2^*$	$20 \pm 6^*$
Healthy control of Type 1 diabetes		34 ± 8	30 ± 7

Table 4.1b- The results of age distribution in the study group for Type 2 diabetics according to the sex.

Variable		Age(years)	
Type 2 diabetics	Diabetic peripheral neuropathy patients	Male 54.6±2.9	Female 53.1±1.8
	Diabetics without neuropathy	49.5±7*	50±4*
Healthy control of Type 2 diabetes		58±4	55±6

Values are mean ± standard error

* Significant at $p < 0.05$ between diabetic peripheral neuropathy patients & diabetics without neuropathy

4.2 Gender differences:-

The gender differences of persons enrolled in the study were as follows:-
For patients with Type 1 DM who have DPN there were 15 male and 20 female, for diabetics without neuropathy there are 5 males and 10 females. For patients with Type 2 DM who had DPN there are 22 male and 48 female, for diabetics without neuropathy there were 12 males and 33 females. For normal healthy controls of Type 1 DM there were 13 male & 9 females & those controls for Type 2 DM there were 20 males & 18 females. These values are shown in Table 4.2. So, of 54 male diabetic patients enrolled in the study there were 37 (69%) affected by DPN, while of 111 female diabetic enrolled in the study there were 68 (61%) affected by DPN. These results indicated higher prevalence of DPN in males than females. This result is shown in Table 4.3.

Table 4.2 Gender differences among study groups.

Variable	Diabetic peripheral neuropathy patients		Diabetic without neuropathy		Healthy controls		Total
	Type 1	Type 2	Type 1	Type 2	Type1	Type2	
males	15	22	5	12	13	20	87
Females	20	48	10	33	9	18	138
Total	35	70	15	45	22	38	225

Table 4.3 Gender differences between diabetic peripheral neuropathy & diabetics without neuropathy patients.

Variable	Developed Diabetic peripheral neuropathy		Diabetics without neuropath		Total
	Number	percentage	Number	percentage	
Males	37	69%	17	31%	54
Females	68	61%	43	39%	111
Total	105		60		

4.3 Types of Diabetes mellitus:-

The patients with DPN are divided into 2 groups according to the type of DM, with 35 (33%) patients that have Type 1 DM and 70 (67%) patients have Type 2 DM. For diabetics without neuropathy there are 15 (25%) patients with Type 1 DM and 45 (75%) patients with Type 2 DM. These values are shown in Figure 4.1. According to the results of study, of 50 patients with type 1 DM there are 35 (70%) have DPN and for patients with

type 2 DM, of 115 patients enrolled in the study there are 70 (60%) have DPN. So the study indicates higher prevalence of DPN in type 1 diabetics.

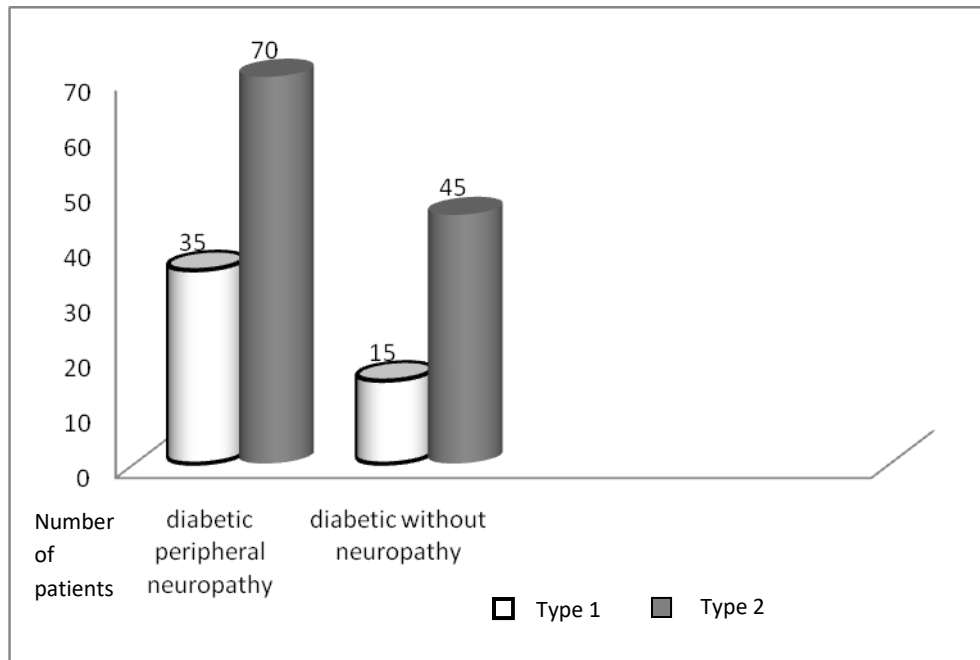


Figure 4.1 the distribution of diabetic patients according to the types of Diabetes mellitus.

4.4 Duration of diabetes mellitus:-

The duration of DM (in years) of patients with Type 1 DM who was presented with diabetic peripheral neuropathy was 14.4 ± 2 years for males and 13.4 ± 2 years for females, for diabetics without neuropathy was 2 ± 2 years for males and 3 ± 2 years for females. The duration of DM (in years) of patients with Type 2 DM who was presented with diabetic peripheral neuropathy was 10.3 ± 2 years for males and 8.2 ± 1 years for females, for diabetics without neuropathy was 3.5 ± 3 years for males and 2.2 ± 3 years for females. There are significant differences between diabetic peripheral

neuropathy patients and diabetic without neuropathy control ($P < 0.05$). These values are shown in Table 4.4.

Table 4.4 The duration of diabetes mellitus of diabetic peripheral neuropathy and diabetic without neuropathy according to the type of diabetes and sex.

Variable		Duration(years)	
Type 1 diabetics	Diabetic peripheral neuropathy	Male 14.4±2	Female 13.4±2
	Diabetics without neuropathy	2±2*	3±2*
Type 2 diabetics	Diabetic peripheral neuropathy	10.3±2	8.2±1
	Diabetics without neuropathy	3.5±3*	2.2±3*

Values are mean ± standard error

* Significant at $p < 0.05$

4.4.1 Distribution of diabetic patients according to the duration of diabetes:-

Diabetic peripheral neuropathy is more prevalent in patients with longer duration of DM and so patients with DM duration <5 years represent 19% (20 patients) of the study group, between 5-10 years 25% (27), 10-20 years 35% (37) and > 20 years constitute 20% (21) of the study group, while most of control group (diabetic without neuropathy) have short duration of DM with 91% (55) of controls (diabetic without neuropathy) have duration <5 years. These results are shown in Figure 4.2.

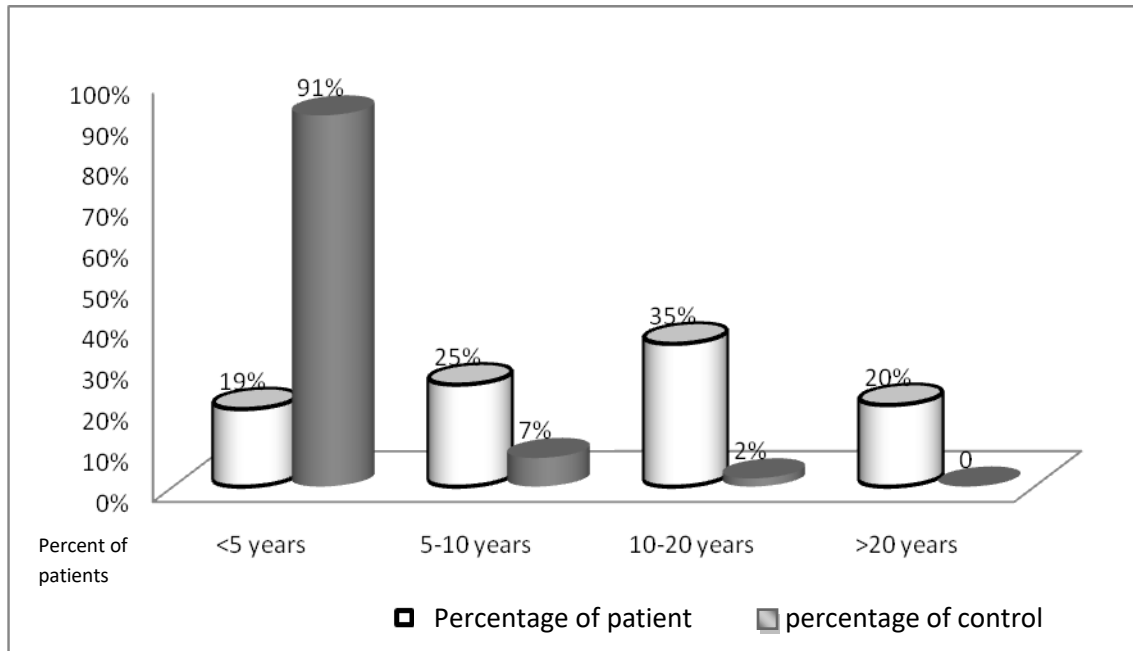


Figure 4.2 the distribution of diabetic patients according to the duration of diabetes mellitus.

4.5 Clinical features of diabetic peripheral neuropathy:-

4.5.1 Symptoms of neuropathy

Parasthesia of the distal parts of the limbs (fingers and toes) was the main symptom and from the total number of patients it was seen in 91 patients (84% of the DPN patients) followed by pain of muscles or burning pain of distal extremities seen in 53 patients (50% of DPN patients) and weakness of proximal muscles like difficulty in standing from squatting position or getting upstairs seen in 13 patients (12% of DPN patients). These results are illustrated in Table 4.5.

4.5.2 Signs:-

The most common and early abnormality found during neurological examination were reduced vibration perception threshold of the big toe and medial malleolus which was seen in 81 patients (77% of DPN patients). Reduced deep tendon reflexes of ankle and less commonly other joints were

seen in 74 patients (70% of DPN patients). Impaired pinprick sensation of distal parts of body (stock and glove distribution) was seen in 73 patients (69% of DPN patients). Reduced power of upper or lower limbs (power grade 3 and 4) were seen in 11 patients (10% of DPN patients). These values are shown in Table 4.5.

Table 4.5 the prevalence of each clinical feature in patients with DPN.

Clinical feature		Number and percentage of patients	
		Number	Percentage
Symptoms	Paresthesia	91	84%
	Pain	53	50%
	Weakness	13	12%
Signs	Decrease vibration	81	77%
	Reduced reflexes	74	70%
	Decrease sensation	73	69%
	Decrease power	11	10%

These symptoms and signs do not involve all the limbs at the same time, so the symptoms and signs may involve either lower or upper limbs or both at the same time, also they may be bilateral or unilateral. These variations are explained in Table 4.6 (a & b).

Table 4.6a The anatomical distribution of clinical features in patients.

Variable	Lower limb	Upper limbs	Total patients
Paresthesia	77	45	91
Pain	38	15	53
Weakness	13	2	13
Vibration	79	22	81
Reflexes	70	20	74
Sensation	61	18	73
Power	11	2	11

Table 4.6b- The anatomical distribution of clinical features in diabetic peripheral neuropathy patients according to the side of involvement.

Variable	Bilateral (both sides)		Unilateral (one side)		Total patients
	number	percent	number	Percent	
Paresthesia	69	75%	22	25%	91
Pain	50	95%	3	5%	53
Weakness	11	98%	2	2%	13
Vibration	71	90%	10	10%	81
Reflexes	67	89%	7	11%	74
Sensation	57	88%	6	12%	73
Power	10	98%	1	2%	11

4.6 Fasting blood sugar (FBS):-

The fasting blood sugar (FBS) level in millimol/liter of patients with Type 1 DM who were presented with diabetic peripheral neuropathy was 12.2 ± 1 mmol/L for males and 13.2 ± 1 mmol/L for females, for diabetics without neuropathy was 7 ± 2 mmol/L for males and 8 ± 2 mmol/L for females. The FBS value of patients with Type 2 DM who were presented with diabetic peripheral neuropathy was 12.8 ± 1 mmol/L for males and 12.5 ± 1 mmol/L for females, for diabetics without neuropathy was 8 ± 4 mmol/L for males and 8.2 ± 2 mmol/L for females. The FBS value of normal healthy control of Type 1 DM was 6.2 ± 2 mmol/L for males and 5.9 ± 2 mmol/L for females and for Type 2 DM was 5.2 ± 2 mmol/L for males and 5.7 ± 1 mmol/L for females. There are significant differences between DPN patients and diabetics without neuropathy at $p < 0.05$ and DPN with normal healthy controls at $p < 0.001$. These values are shown in Table 4.7 (a & b).

4.7 Glycosylated Hemoglobin (HbA1c):-

The glycosylated hemoglobin (HbA1c) level percentage of patients with Type 1 DM who were presented with diabetic peripheral neuropathy was $8.2 \pm 0.7\%$ for males and $10.5 \pm 1\%$ for females, for diabetics without neuropathy was $5.6 \pm 1\%$ for males and $5 \pm 1\%$ for females. The HbA1c level of patients with Type 2 DM who were presented with diabetic peripheral neuropathy was $8.7 \pm 0.8\%$ for males and $8.6 \pm 0.4\%$ for females, for diabetics without neuropathy was $7.2 \pm 1\%$ for males and $5.3 \pm 1\%$ for females. The HbA1c level of normal healthy control for Type 1 DM was $4.5 \pm 2\%$ for males and $5 \pm 0.7\%$ for females and for Type 2 DM was $4.3 \pm 1\%$ for males and $4 \pm 1\%$ for females. There are significant differences between DPN patients and diabetics without neuropathy at $p < 0.05$ and DPN with normal healthy controls at $p < 0.001$. These values are shown in Table 4.7 (a & b).

Table 4.7a- The values of fasting blood sugar and Glycosylated hemoglobin between patients and controls of Type 1 diabetics according to the sex.

Variable		Fasting blood sugar (mmol/L)		Glycosylated hemoglobin %	
Type 1 Diabetics	Diabetic peripheral neuropathy patients	Male	Female	Male	Female
		12.2 ± 1	13.2 ± 1	8.2 ± 0.7	10.5 ± 1
	Diabetics without neuropathy	$7 \pm 2^*$	$8 \pm 2^*$	$5.6 \pm 1^*$	$5 \pm 1^*$
Healthy control of Type 1 diabetics		$6.2 \pm 2^{\dagger\dagger}$	$5.9 \pm 2^{\dagger\dagger}$	$4.5 \pm 2^{\dagger\dagger}$	$5 \pm 0.7^{\dagger\dagger}$

Table 4.7b The values of fasting blood sugar and Glycosylated hemoglobin between patients and controls of Type 2 diabetics according to the sex.

Variable		Fasting blood sugar (mmol/L)		Glycosylated hemoglobin %	
Type 2 Diabetics		Male	Female	Male	Female
	Diabetic peripheral neuropathy	12.8±1	12.5±1	8.7±0.8	8.6±0.4
	Diabetics without neuropathy	8±4*	8.2±2*	7.2±1*	5.3±1*
Healthy controls of Type 2 diabetics		5.2±2††	5.7±1††	4.3±1††	4±1††

Values are mean ± standard error

*, ** Significant differences at (*= p<0.05) & (**=p<0.001) between DPN & diabetics without neuropathy control. †, †† Significant differences at (†= p<0.05) &

(††=p<0.001) between DPN & healthy control

4.8 Lipid profile abnormalities:-

4.8.1 Total cholesterol (TC):-

The total cholesterol level in of patients with Type 1 DM who were presented with DPN was 4.5±0.3 mmol/L for males and 4.3±0.4 mmol/L for females, for diabetics without neuropathy was 4±0.6 mmol/L for males and 4±0.3 mmol/L for females. The TC level of patients with Type 2 DM who were presented with DPN was 3.7±0.4 mmol/L for males and 5.1±0.2 mmol/L for females, for diabetics without neuropathy was 4.3±0.7 mmol/L for males and 4.3±1 mmol/L for females. The TC level of normal healthy control group of Type 1 DM was 4.2±0.4 mmol/L for males and 4±1 mmol/L for females & for healthy controls of Type 2 DM was 4.2±1 mmol/L for males & 4.5±1.1 mmol/L for females. There are no significant differences between patients and control groups. These values are shown in Table 4.8 (a & b).

4.8.2 Triglycerides (TG):-

The triglyceride level in of patients with Type 1 DM who were presented with DPN was 1.3 ± 0.4 mmol/L for males and 0.8 ± 0.6 mmol/L for females, for diabetics without neuropathy was 1.1 ± 1 mmol/L for males and 1 ± 0.4 mmol/L for females. The TG level of patients with Type 2 DM who were presented with DPN was 1.1 ± 0.6 mmol/L for males and 1.3 ± 0.2 mmol/L for females, for diabetics without neuropathy was 0.8 ± 0.6 mmol/L for males and 1.1 ± 0.6 mmol/L for females. The TG level of normal healthy control group of Type 1 DM was 1 ± 0.4 mmol/L for males and 1.2 ± 0.3 mmol/L for females & for healthy controls of Type 2 DM was 0.8 ± 0.2 mmol/L for males & 0.7 ± 0.5 mmol/L for females. There are no significant differences between patients and control. These values are shown in Table 4.8 (a & b).

4.8.3 Very Low Density Lipoprotein (VLDL):-

The VLDL level in of patients with Type 1 DM who were presented with DPN was 0.2 ± 0.08 mmol/L for males and 0.17 ± 0.1 mmol/L for females, for diabetics without neuropathy was 0.1 ± 0.03 mmol/L for males and 0.15 ± 0.1 mmol/L for females. The VLDL level of patients with Type 2 DM who were presented with DPN was 0.22 ± 0.1 mmol/L for males and 0.2 ± 0.05 mmol/L for females, for diabetics without neuropathy was 0.16 ± 0.1 mmol/L for males and 0.2 ± 0.07 mmol/L for females. The VLDL level of normal healthy control group of patients with Type 1 DM was 0.27 ± 0.1 mmol/L for males and 0.2 ± 0.2 mmol/L for females & for the persons with Type 2 DM was 0.13 ± 0.1 mmol/L for males & 0.2 ± 0.1 mmol/L for females. There are no significant differences between patients and controls. These values are shown in Table 4.8 (a & b).

4.8.4 High Density Lipoprotein (HDL):-

The HDL level of patients with Type 1 DM who were presented with DPN was 0.9 ± 0.1 mmol/L for males and 1.8 ± 0.2 mmol/L for females, for diabetics without neuropathy was 1 ± 0.6 mmol/L for males and 1.3 ± 0.4 mmol/L for females. The HDL level of patients with Type 2 DM who were presented with DPN was 1.1 ± 0.2 mmol/L for males and 1.4 ± 0.1 mmol/L for females, for diabetics without neuropathy was 0.6 ± 0.2 mmol/L for males and 1 ± 0.1 mmol/L for females. The HDL level of normal healthy control group of patients with Type 1 DM was 1.2 ± 0.6 mmol/L for males and 1.5 ± 0.9 mmol/L for females & for patients with Type 2 DM was 1.3 ± 0.6 mmol/L for males & 2 ± 1.1 mmol/L for females. There are no significant differences between patients and controls. These values are shown in Table 4.8 (a & b).

4.8.5 Low Density Lipoprotein (LDL):-

The LDL level in of patients with Type 1 DM who were presented with DPN was 2.9 ± 0.8 mmol/L for males and 3 ± 1 mmol/L for females, for diabetics without neuropathy was 2.5 ± 1 mmol/L for males and 2.8 ± 2 mmol/L for females. The LDL level of patients with Type 2 DM who were presented with DPN was 2.3 ± 1 mmol/L for males and 3.1 ± 0.5 mmol/L for females, for diabetics without neuropathy was 2.8 ± 1 mmol/L for males and 2.6 ± 0.6 mmol/L for females. The LDL level of normal healthy control group of Type 1 DM was 2 ± 1.2 mmol/L for males and 2.1 ± 1 mmol/L for females & for patients with Type 2 DM was 2.3 ± 1 mmol/L for males & 2.8 ± 2 mmol/L for females. There are no significant differences between patients and controls. These values are shown in Table 4.8 (a & b).

Table 4.8a The values of lipid profile between patients & controls of type 1 diabetics according to the sex.

Variable	TC (mmol/L)		TG (mmol/L)		VLDL (mmol/L)		HDL (mmol/L)		LDL (mmol/L)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Type 1 DM										
DPN patients	4.5±0.3	4.3±0.4	1.3±0.4	0.8±0.6	0.2±0.08	0.17±0.1	0.9±0.1	1.8±0.2	2.9±0.8	3±1
Diabetics without neuropathy	4±0.6	4±0.3	1.1±1	1±0.4	0.1±0.03	0.15±0.1	1±0.6	1.3±0.4	2.5±1	2.8±2
Healthy control of type 1 DM	4.2±0.4	4±1	1±0.4	1.2±0.3	0.27±0.1	0.2±0.2	1.2±0.6	1.5±0.9	2±1.2	2.1±1

Table 4.8b- The values of lipid profile between patients & controls of type 2 diabetics according to the sex.

Variable	TC (mmol/L)		TG (mmol/L)		VLDL (mmol/L)		HDL (mmol/L)		LDL (mmol/L)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Type 2 DM										
DPN patients	3.7±0.4	5.1±0.2	1.1±0.6	1.3±0.2	0.22±0.1	0.2±0.05	1.1±0.2	1.4±0.1	2.3±1	3.1±0.5
Diabetics without neuropathy	4.3±0.7	4.3±1	0.8±0.6	1.1±0.6	0.16±0.1	0.2±0.07	0.6±0.2	1±0.1	2.8±1	2.6±0.6
Healthy control of type 2 DM	4.2±1	4.5±1.1	0.8±0.2	0.7±0.5	0.13±0.1	0.2±0.1	1.3±0.6	2±1.1	2.3±1	2.8±2

Values are mean ± standard error, results are not significant. DPN: diabetic peripheral neuropathy, DM: diabetes mellitus, TC: total cholesterol, TG: triglyceride, VLDL: very low density lipoprotein, HDL: high density lipoprotein, LDL: low density lipoprotein.

4.9 Serum selenium level:-

The serum selenium (SS) level in milligram/liter of patients with Type 1 DM who were presented with diabetic peripheral neuropathy was 61 ± 5 mg/L for males and 58 ± 8 mg/L for females, for diabetics without neuropathy was 70 ± 4 mg/L for males and 74 ± 5 mg/L for females. The SS level of patients with Type 2 DM who were presented with diabetic peripheral neuropathy was 44 ± 8 mg/L for males and 62 ± 3 mg/L for females, for diabetics without neuropathy was 63 ± 14 mg/L for males and 69 ± 10 mg/L for females. The SS level of normal healthy control of Type 1 DM was 97 ± 6 mg/L for males and 90 ± 5 mg/L for females and of Type 2 DM was 88 ± 6 mg/L for males and 99 ± 4 mg/L for females. There are significant differences between DPN patients and diabetics without neuropathy at $p < 0.05$ and DPN with normal healthy controls at $p < 0.001$. These values are shown in Table 4.9 (a & b).

4.10 Vitamin E level:-

The serum vitamin E level in millimol/liter of patients with Type 1 DM who were presented with diabetic peripheral neuropathy was 2.7 ± 0.4 mmol/L for males and 1.8 ± 0.4 mmol/L for females, for diabetics without neuropathy was 4 ± 0.3 mmol/L for males and 4.5 ± 1 mmol/L for females. The serum vitamin E level of patients with Type 2 DM who were presented with diabetic peripheral neuropathy was 1.7 ± 0.7 mmol/L for males and 2.5 ± 0.4 mmol/L for females, for diabetics without neuropathy was 4.2 ± 0.2 mmol/L for males and 3.9 ± 1 mmol/L for females. The serum vitamin E level of normal healthy control of Type 1 DM was 7.3 ± 3.5 mmol/L for males and 7.5 ± 1.9 mmol/L for females and of Type 2 DM was 7.9 ± 2 mmol/L for males and 6.9 ± 3 mmol/L for females. There are significant

differences between DPN patients and diabetics without neuropathy at $p < 0.05$ and DPN with normal healthy controls at $p < 0.001$. These values are shown in Table 4.9 (a & b).

Table 4.9a- The selenium & vitamin E levels in patients & controls of Type 1 diabetics according to the sex.

Variable		Selenium(mg/L)		Vitamin E(mmol/L)	
Type 1 Diabetics		Male	Female	Male	Female
	Diabetic peripheral neuropathy	61±5	58±8	2.7±0.4	1.8±0.4
	Diabetics without neuropathy	70±4*	74±5*	4±0.3*	4.5±1*
Healthy control of Type 1 diabetics		97±6††	90±5††	7.3±3.5††	7.5±1.9††

Table 4.9b- The selenium & vitamin E levels in patients & controls of Type 2 diabetics according to the sex.

Variable		Selenium(mg/L)		Vitamin E(mmol/L)	
Type 2 Diabetics		Male	Female	Male	Female
	Diabetic peripheral neuropathy	44±8	62±3	1.7±0.7	2.5±0.4
	Diabetics without neuropathy	63±14*	69±10*	4.2±0.2*	3.9±1*
Healthy controls of Type 2 diabetics		88±6††	99±4††	7.9±2††	6.9±3††

Values are mean ± standard error

* Significant differences at ($p < 0.05$) between DPN & diabetics without neuropathy control

†† Significant differences at ($p < 0.001$) between DPN & healthy control

4.11 Electrophysiological assessment:-

The results of electrophysiological test of study group were as following:-

4.11.1 Median nerve:-

4.11.1.1 Results of motor nerve conduction study:

The results of motor nerve conduction study of median nerve for patients with Type 1 DM who were presented with DPN were as follows: motor latency (DML) in millisecond, amplitude (CMAP) in millivolt and conduction velocity (CV) in meter/second which show the following results respectively, 4.4 ± 0.3 ms, 11 ± 1 mv and 48 ± 2 m/s for males and 5 ± 0.3 ms, 7 ± 1 mv and 45 ± 2 m/s for females, while for diabetics without neuropathy were as follows: 2.8 ± 1 ms, 5 ± 2 mv and 49 ± 1 m/s for males and 2.4 ± 1 ms, 7 ± 1 mv and 50 ± 1 m/s for females. For patients with Type 2 DM who were presented with DPN the results of motor nerve conduction study were as following, motor latency, amplitude and conduction velocity show the following results respectively, 4.3 ± 0.2 ms, 12 ± 1 mv and 42 ± 2 m/s for males and 4.3 ± 0.5 ms, 9 ± 0.8 mv and 47 ± 1 m/s for females, while for diabetics without neuropathy, 3 ± 1 ms, 9 ± 1 mv and 50 ± 1 m/s for males and 3.3 ± 0.5 ms, 7 ± 2 mv and 57 ± 2 m/s for females. For normal healthy controls of Type 1 DM, the results were as follows: 2.3 ± 3 ms, 12 ± 4 mv and 56 ± 6 m/s for males and 2.1 ± 2 ms, 20 ± 2 mv and 57 ± 3 m/s for females and of Type 2 DM, the results were as follows: 2.2 ± 4 ms, 14 ± 4 mv and 59 ± 9 m/s for males and 2.2 ± 1 ms, 23 ± 11 mv and 66 ± 2 m/s for females. There are statistically significant differences between DPN patients and diabetics without neuropathy regarding motor latency and conduction velocity at $p < 0.05$ and DPN with normal healthy controls regarding motor latency and conduction

velocity at $p<0.001$ and regarding amplitude at $p<0.05$. These results are shown in Table 4.10 (a & b).

Table 4.10a- The electrophysiological of median nerve (motor part) in patients and control groups of Type 1 diabetics according to the sex.

Variable		Distal motor latency (ms)		Compound muscle action potential (mv)		Conduction velocity(m/s)	
Type 1 Diabetics		Male	Female	Male	female	Male	female
	Diabetic peripheral neuropathy	4.4±0.3	5±0.3	11±1	7±1	48±2	45±2
	Diabetics without neuropathy	2.8±1*	2.4±1*	5±2	7±1	49±1*	50±1*
Healthy control of Type 1 diabetics		2.3±3††	2.1±2††	12±4†	20±2†	56±6††	57±3††

Table 4.10b- The electrophysiological of median nerve (motor part) in patients and control groups of Type 2 diabetics according to the sex.

Variable		Distal motor latency (ms)		Compound muscle action potential (mv)		Conduction velocity(m/s)	
Type 2 Diabetics		Male	Female	Male	female	Male	female
	Diabetic peripheral neuropathy	4.3±0.2	4.3±0.5	12±1	9±0.8	42±2	47±1
	Diabetics without neuropathy	3±1*	3.3±0.5*	9±1	7±2	50±1*	57±2*
Healthy controls of Type 2 diabetics		2.2±4††	2.2±1††	14±4†	23±11†	59±9††	66±2††

Values are mean ± standard error.

* Significant differences at ($p<0.05$) between DPN & diabetics without neuropathy control

†, †† Significant differences at (†= $p<0.05$) & (††= $p<0.001$) between DPN & healthy control

4.11.1.2 Results of sensory nerve conduction study of median nerve: -

The results of sensory nerve conduction study for patients with Type 1 DM who were presented with DPN were as follows: sensory latency (DSL) and amplitude (SNAP) which show the following results respectively, 3.6 ± 1 ms and 40 ± 14 μ v for males and 3.2 ± 1 ms and 37 ± 14 μ v for females, while for diabetics without neuropathy, 2.3 ± 1 ms and 90 ± 11 μ v for males and 2.2 ± 3 ms and 115 ± 32 μ v for females. For patients with Type 2 DM who were presented with DPN, sensory latency and amplitude show the following results respectively, 3.9 ± 1 ms and 38 ± 16 μ v for males and 4.2 ± 0.6 ms and 44 ± 8 μ v for females, while for diabetics without neuropathy, 2.4 ± 1 ms and 100 ± 21 μ v for males and 2.6 ± 1 ms and 126 ± 22 μ v for females. For normal healthy controls of Type 1 DM controls were as follows: 2 ± 3 ms and 149 ± 21 μ v for males and 2.3 ± 1 ms and 66 ± 2 μ v for females and of Type 2 DM controls were as follows: 3 ± 2 ms and 144 ± 24 μ v for males and 2.1 ± 2 ms and 76 ± 2 μ v for females. There are statistically significant differences between DPN patients and diabetics without neuropathy and DPN patients with normal healthy controls regarding sensory latency and amplitude at $p < 0.05$. These results are shown in Table 4.11 (a & b).

Table 4.11a- The electrophysiological of median nerve (sensory part) in patients and control groups of Type 1 diabetics according to the sex.

Variable		Distal sensory latency (ms)		Sensory nerve action potential(μ v)	
Type 1 Diabetics	Diabetic peripheral neuropathy	Male	Female	Male	Female
	Diabetics without neuropathy	$2.3 \pm 1^*$	$2.2 \pm 3^*$	$90 \pm 11^*$	$115 \pm 32^*$
Healthy control of Type 1 diabetics		$2 \pm 3^\dagger$	$2.3 \pm 1^\dagger$	$149 \pm 21^\dagger$	$66 \pm 2^\dagger$

Table 4.1b- The electrophysiological of median nerve (sensory part) in patients and control groups of Type 2 diabetics according to the sex.

Variable		Distal sensory latency (ms)		Sensory nerve action potential(μ V)	
Type 2 Diabetics		Male	Female	Male	Female
	Diabetic peripheral neuropathy	3.9 $\pm$ 1	4.2 $\pm$ 0.6	38 $\pm$ 16	44 $\pm$ 8
	Diabetics without neuropathy	2.4 $\pm$ 1*	2.6 $\pm$ 1*	100 $\pm$ 21*	126 $\pm$ 22*
Healthy controls of Type 2 diabetics		3 $\pm$ 2†	2.1 $\pm$ 2†	144 $\pm$ 24†	76 $\pm$ 2†

Values are mean $\pm$ standard error.

* Significant differences at (*= p<0.05) between patients & diabetics without neuropathy control.

† Significant differences at (†= p<0.05) between patients & healthy control

4.11.2 Ulnar nerve:-

4.11.2.1 Motor nerve conduction study:-

For patients with Type 1 DM who were presented with DPN, motor latency (DML) in millisecond, amplitude (CMAP) in millivolt and conduction velocity (CV) in meter/second show the following results respectively, 3.3 $\pm$ 0.2 ms, 7.4 $\pm$ 1 mv and 46 $\pm$ 2 m/s for males and 2.7 $\pm$ 0.2 ms, 7.2 $\pm$ 1 mv and 47 $\pm$ 2 m/s for females, while for diabetics without neuropathy, 2.2 $\pm$ 0.6 ms, 12 $\pm$ 1 mv and 50 $\pm$ 1 m/s for males and 2.3 $\pm$ 0.8 ms, 11 $\pm$ 2 mv and 54 $\pm$ 6 m/s for females. For patients with Type 2 DM who were presented with DPN, motor latency, amplitude and conduction velocity show the following results respectively, 3.2 $\pm$ 0.2 ms, 5.1 $\pm$ 0.9 mv and 42 $\pm$ 2 m/s for males and 3 $\pm$ 0.1 ms, 6.2 $\pm$ 0.5 mv and 47 $\pm$ 1 m/s for females, while for diabetics without neuropathy, 2.3 $\pm$ 0.4 ms, 10 $\pm$ 1 mv and 55 $\pm$ 2 m/s for males

and 2.8 ± 0.8 ms, 9 ± 1 mv and 61 ± 2 m/s for females. For normal healthy controls of Type 1 DM was as follows: 2 ± 0.1 ms, 17 ± 1 mv and 61 ± 7 m/s for males and 2.1 ± 0.2 ms, 15 ± 2 mv and 58 ± 1 m/s for females and For normal healthy controls of Type 2 DM was as follows: 2 ± 0.3 ms, 13 ± 2 mv and 64 ± 7 m/s for males and 1.9 ± 0.2 ms, 15 ± 2 mv and 56 ± 4 m/s for females. There are statistically significant differences between DPN patients and diabetics without neuropathy regarding amplitude and conduction velocity at $p < 0.05$ and DPN with normal healthy controls regarding motor latency, amplitude and conduction velocity at $p < 0.001$. These results are shown in Table 4.12 (a & b).

Table 4.12a- The electrophysiological of ulnar nerve (motor part) in patients and control groups of Type 1 diabetics according to the sex.

Variable		Distal motor latency (ms)		Compound muscle action potential (mv)		Conduction velocity(m/s)	
Type 1 Diabetics		Male	Female	Male	female	Male	female
	Diabetic peripheral neuropathy	3.3 ± 0.2	2.7 ± 0.2	7.4 ± 1	7.2 ± 1	46 ± 2	47 ± 2
	Diabetics without neuropathy	2.2 ± 0.6	2.3 ± 0.8	$12 \pm 1^*$	$11 \pm 2^*$	$50 \pm 1^*$	$54 \pm 6^*$
Healthy control of Type 1 diabetics		$2 \pm 0.1^{\dagger\dagger}$	$2.1 \pm 0.2^{\dagger\dagger}$	$17 \pm 1^{\dagger\dagger}$	$17 \pm 2^{\dagger\dagger}$	$61 \pm 7^{\dagger\dagger}$	$58 \pm 1^{\dagger\dagger}$

Table 4.12b- The electrophysiological of ulnar nerve (motor part) in patients and control groups of Type 2 diabetics according to the sex.

variable		Distal motor latency (ms)		Compound muscle action potential (mv)		Conduction velocity(m/s)	
Type 2 Diabetics		Male	Female	Male	female	Male	female
	Diabetic peripheral neuropathy	3.2±0.2	3±0.1	5.1±0.9	6.2±0.5	42±2	47±1
	Diabetics without neuropathy	2.3±0.4	2.8±0.8	10±1*	9±1*	55±2*	61±2*
Healthy controls of Type 2 diabetics		2±0.3††	1.9±0.2††	13±2††	15±2††	64±7††	56±4††

Values are mean ± standard error.

*Significant differences at (p<0.05) between patients & diabetics without neuropathy control

†† Significant differences at (p<0.001) between patients & healthy control

4.11.2.2 Results of sensory nerve conduction study of ulnar nerve: -

For patients with Type 1 DM who were presented with DPN, sensory latency (DSL) in millisecond and amplitude (SNAP) microvolt show the following results respectively, 3.3±0.7 ms and 83±7 µv for males and 3.2±0.7 ms and 74±4 µv for females, while for diabetics without neuropathy, 2.1±0.2 ms and 111±3 µv for males and 2±2 ms and 114±3 µv for females. For patients with Type 2 DM who were presented with DPN, sensory latency and amplitude show the following results respectively, 4±0.9 ms and 85±4 µv for males and 3.7±0.4 ms and 81±16 µv for females, while for diabetics without neuropathy, 2.2±1 ms and 113±4 µv for males and 2.2±1 ms and 111±32 µv for females. For normal healthy controls of Type 1 DM was as follows: 2.1±2 ms and 126±40 µv for males and 2.2±1 ms and 123±60 µv for females and for normal healthy controls of Type 2 DM was

as follows: 2.2 ± 3 ms and 111 ± 40 μ v for males and 2.3 ± 2 ms and 122 ± 65 μ v for females. There are statistically significant differences between DPN patients and diabetics without neuropathy and DPN patients with normal healthy control regarding sensory latency at $p < 0.05$. These results are shown in Table 4.13 (a & b).

Table 4.13a- The electrophysiology of ulnar nerve (sensory part) in patients and controls of Type 1 diabetics according to the sex.

Variable		Distal sensory latency (ms)		Sensory nerve action potential(μ v)	
Type 1 Diabetics		Male	Female	Male	female
	Diabetic peripheral neuropathy	3.3 ± 0.7	3.2 ± 0.7	83 ± 7	74 ± 4
	Diabetics without neuropathy	$2.1 \pm 0.2^*$	$2 \pm 2^*$	111 ± 3	114 ± 3
Healthy control of Type 1 diabetics		$2.1 \pm 2^\dagger$	$2.2 \pm 1^\dagger$	126 ± 40	123 ± 60

Table 4.13b- The electrophysiology of ulnar nerve (sensory part) in patients and controls of Type 2 diabetics according to the sex.

Variable		Distal sensory latency (ms)		Sensory nerve action potential(μ v)	
Type 2 Diabetics		Male	Female	Male	Female
	Diabetic peripheral neuropathy	4 ± 0.9	3.7 ± 0.4	85 ± 4	81 ± 16
	Diabetics without neuropathy	$2.2 \pm 1^*$	$2.2 \pm 1^*$	113 ± 4	111 ± 32
Healthy controls of Type 2 diabetics		$2.2 \pm 3^\dagger$	$2.3 \pm 2^\dagger$	111 ± 40	122 ± 65

Values are mean $\pm$ standard error.

*Significant differences at ($= p < 0.05$) between patients & diabetics without neuropathy control.

† Significant differences at ($^\dagger = p < 0.05$) between patients & healthy control.

4.11.3 Common peroneal nerve: -

Common peroneal nerve is a motor nerve & so it is assessed by motor nerve conduction study. For patients with Type 1 DM who were presented with DPN, motor latency (DML) in millisecond, amplitude (CMAP) in millivolt and conduction velocity (CV) in meter/second show the following results respectively, 5.3 ± 0.5 ms, 11 ± 3 mv and 36 ± 3 m/s for males and 3.5 ± 0.5 ms, 13 ± 2 mv and 30 ± 3 m/s for females, while for diabetics without neuropathy, 3.4 ± 1 ms, 21 ± 2 mv and 40 ± 2 m/s for males and 3.6 ± 1 ms, 29 ± 3 mv and 42 ± 1 m/s for females. For patients with Type 2 DM who were presented with DPN, motor latency, amplitude and conduction velocity show the following results respectively, 3.8 ± 0.6 ms, 14 ± 3 mv and 25 ± 2 m/s for males and 4.6 ± 0.3 ms, 31 ± 9 mv and 36 ± 2 m/s for females, while for diabetics without neuropathy, 4 ± 1 ms, 33 ± 1 mv and 44 ± 5 m/s for males and 3 ± 1 ms, 44 ± 2 mv and 51 ± 5 m/s for females. For normal healthy controls of Type 1 DM was as follows: 3.2 ± 1 ms, 45 ± 70 mv and 47 ± 11 m/s for males and 3.3 ± 0.8 ms, 40 ± 30 mv and 52 ± 7 m/s for females and for normal healthy controls of Type 2 DM was as follows: 3.3 ± 2 ms, 49 ± 60 mv and 42 ± 12 m/s for males and 3.2 ± 1 ms, 43 ± 33 mv and 53 ± 11 m/s for females. There are statistically significant differences between DPN patients and diabetics without neuropathy and DPN with normal healthy controls regarding conduction velocity at $p < 0.05$. These results are shown in Table 4.14 (a & b).

Table 4.14a- The electrophysiology of common peroneal nerve in patients and controls of Type 1 diabetics according to the sex.

Variable		Distal motor latency (ms)		Compound muscle action potential (mv)		Conduction velocity(m/s)	
Type 1 Diabetics		Male	Female	Male	female	Male	female
	Diabetic peripheral neuropathy	5.3±0.5	3.5±0.5	11±3	13±2	36±3	30±3
	Diabetics without neuropathy	3.4±1	3.6±1	21±2	29±3	40±2*	42±1*
Healthy control of Type 1 diabetics		3.2±1	3.3±0.8	45±70	40±30	47±11†	52±7†

Table 4.14b- The electrophysiology of common peroneal nerve in patients and controls of Type 2 diabetics according to the sex.

Variable		Distal motor latency (ms)		Compound muscle action potential (mv)		Conduction velocity(m/s)	
Type 2 Diabetics		Male	Female	Male	female	Male	female
	Diabetic peripheral neuropathy	3.8±0.6	4.6±0.3	14±3	31±9	25±2	36±2
	Diabetics without neuropathy	4±1	3±1	33±1	44±2	44±5*	51±5*
Healthy controls of Type 2 diabetics		3.3±2	3.2±1	49±60	43±33	42±12†	53±11†

Values are mean ± standard error.

*Significant differences at (p<0.05) between patients & diabetics without neuropathy control

†Significant differences at (p<0.05) between patients & healthy control

4.11.4 Posterior tibial nerve: -

Posterior tibial nerve is a motor nerve & so it is assessed by motor nerve conduction study. For patients with Type 1 DM who were presented with DPN, motor latency (DML) in millisecond, amplitude (CMAP) in millivolt and conduction velocity (CV) meter/second show the following results respectively: 6.5 ± 0.5 ms, 7.2 ± 2 mv and 31.9 ± 3 m/s for males and 4.7 ± 0.5 ms, 6.4 ± 3 mv and 34 ± 3 m/s for females, while for diabetics without neuropathy, 3.3 ± 2 ms, 23 ± 32 mv and 44 ± 5 m/s for males and 3.9 ± 1 ms, 21 ± 32 mv and 52 ± 9 m/s for females. For patients with Type 2 DM who were presented with DPN, motor latency, amplitude and conduction velocity show the following results respectively, 6.8 ± 0.5 ms, 6 ± 21 mv and 31 ± 3 m/s for males and 4.9 ± 0.2 ms, 35 ± 32 mv and 33 ± 1 m/s for females, while for diabetics without neuropathy, 3.9 ± 1 ms, 33 ± 21 mv and 43 ± 6 m/s for males and 4 ± 0.9 ms, 34 ± 23 mv and 45 ± 4 m/s for females. For normal healthy controls of Type 1 DM was as follows, 3.4 ± 1 ms, 45 ± 69 mv and 45 ± 2 m/s for males and 3.4 ± 0.7 ms, 39 ± 50 mv and 46 ± 4 m/s for females and for normal healthy control of Type 2 DM was as follows, 3.1 ± 2 ms, 39 ± 60 mv and 41 ± 6 m/s for males and 3.2 ± 1 ms, 44 ± 52 mv and 49 ± 4 m/s for females. There are statistically significant differences between DPN patients and diabetics without neuropathy regarding amplitude and conduction velocity at $p < 0.05$ and DPN with normal healthy controls regarding motor latency, amplitude and conduction velocity at $p < 0.05$. These results are shown in Table 4.15 (a & b).

Table 4.15a- The electrophysiological changes of posterior tibial nerve in patients and controls of Type 1 diabetics according to the sex.

Variable		Distal motor latency (ms)		Compound muscle action potential (mv)		Conduction velocity(m/s)	
Type 1 Diabetics		Male	Female	Male	Female	Male	female
	Diabetic peripheral neuropathy	6.5±0.5	4.7±0.5	7.2±2	6.4±3	31.9±3	34±3
	Diabetics without neuropathy	3.3±2	3.9±1	23±32*	21±32*	44±5*	52±9*
Healthy control of Type 1 diabetics		3.4±1†	3.4±0.7†	45±69†	39±50†	45±2†	46±4†

Table 4.15 b- The electrophysiological changes of posterior tibial nerve in patients and controls of Type 2 diabetics according to the sex.

Variable		Distal motor latency (ms)		Compound muscle action potential (mv)		Conduction velocity (m/s)	
Type 2 Diabetics		Male	Female	Male	female	Male	female
	Diabetic peripheral neuropathy	6.8±0.5	4.9±0.2	6±21	35±32	31±3	33±1
	Diabetics without neuropathy	3.9±1	4±0.9	33±21*	34±23*	43±6*	45±4*
Healthy control of Type 2 diabetics		3.1±2†	3.2±1†	39±60†	44±52†	41±6†	49±4†

Values are mean ± standard error.

*Significant differences at (p<0.05) between patients & diabetics without neuropathy control

†Significant differences at (p<0.05) between patients & healthy control.

4.11.5 Sural nerve: -

Sural nerve is a sensory nerve & so it is assessed by sensory nerve conduction study. For patients with Type 1 DM who were presented with

DPN, sensory latency (DSL) in millisecond and amplitude (SNAP) in microvolt show the following results respectively, 4 ± 0.3 and 29 ± 7 for males and 3.4 ± 0.3 ms and 20 ± 7 μ v for females, while for diabetics without neuropathy, 2.3 ± 1 ms and 33 ± 3 μ v for males and 2.5 ± 2 ms and 34 ± 6 μ v for females. For patients with Type 2 DM who were presented with DPN, sensory latency and amplitude show the following results respectively, 3.4 ± 0.5 ms and 8 ± 8 μ v for males and 3.4 ± 0.2 ms and 25 ± 4 μ v for females, while for diabetics without neuropathy, 2.1 ± 0.8 ms and 32 ± 2 μ v for males and 2.2 ± 1 ms and 34 ± 5 μ v for females. For normal healthy controls of Type 1 DM was as follows, 2.2 ± 1 ms and 46 ± 23 μ v for males and 2.2 ± 0.5 ms and 37 ± 3 μ v for females and for normal healthy controls of Type 2 DM was as follows, 2 ± 2 and 49 ± 21 for males and 2.3 ± 0.7 ms and 47 ± 13 μ v for females. There are statistically significant differences between DPN patients and diabetics without neuropathy and DPN patients with normal healthy controls regarding sensory latency at $p<0.05$. These results are shown in Table 4.16 (a & b).

Table 4.16a- The electrophysiological changes of sural nerve in patients with Type 1 diabetics and control according to the sex.

Variable		Distal sensory latency (ms)		Sensory nerve action potential(μ v)	
Type 1 Diabetics	Diabetics peripheral neuropathy	Male	Female	Male	Female
		4 ± 0.3	3.4 ± 0.3	29 ± 7	20 ± 7
	Diabetics without neuropathy	$2.3\pm1^*$	$2.5\pm2^*$	33 ± 3	34 ± 6
Healthy control of Type 1 diabetics		$2.2\pm1^\dagger$	$2.2\pm0.5^\dagger$	46 ± 23	37 ± 3

Table 4.16b- The electrophysiological changes of sural nerve in patients with Type 2 diabetics and controls according to the sex.

Variable		Distal sensory latency (ms)		Sensory nerve action potential(μ V)	
Type 2 Diabetics	Diabetic peripheral neuropathy	Male	Female	Male	Female
		3.4 $\pm$ 0.5	3.4 $\pm$ 0.2	8 $\pm$ 8	25 $\pm$ 4
Type 2 Diabetics	Diabetics without neuropathy	Male	Female	Male	Female
		2.1 $\pm$ 0.8*	2.2 $\pm$ 1*	32 $\pm$ 2	34 $\pm$ 5
Healthy control of Type 2 diabetics		Male	Female	Male	Female
		2 $\pm$ 2†	2.3 $\pm$ 0.7†	49 $\pm$ 21	47 $\pm$ 13

Values are mean $\pm$ standard error.

*Significant differences at ($p < 0.05$) between patients & diabetics without neuropathy control

†Significant differences at ($p < 0.05$) between patients & healthy control.

4.11.6 Nerves involvement:-

The most commonly affected nerve was sural nerve which is affected in all patients, followed by median sensory which is affected in 84 patient (81%), common peroneal nerve affected in 44 (42%), posterior tibial affected in 40 patients (37%), Median motor nerve affected in 32 patients (30%). Ulnar sensory nerve affected in 32 patients (30%), Ulnar motor nerve affected in 27 (25%). These results are shown in Figure 4.3.

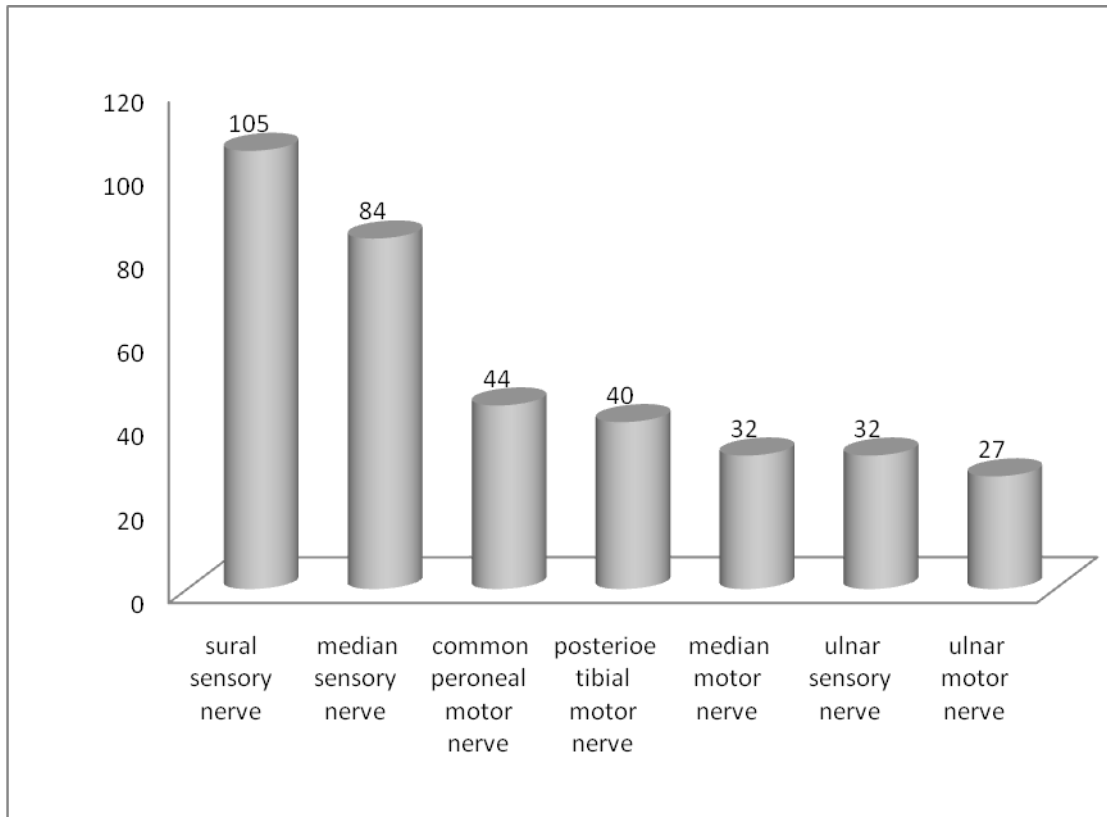


Figure 4.3 The prevalence of abnormal nerves in patients with diabetic peripheral neuropathy.

As well as the results of study shows that sensory nerves affected earlier & more common than motor nerves, so 51 (48%) patients had sensory neuropathy, 54 (52%) patients had mixed motor & sensory neuropathy. Sensory neuropathy was diagnosed by reduction in conduction velocity & amplitude of sensory nerves with normal motor nerves parameters.

According to the pathophysiology, there are 22 patients had developed axonal neuropathy, 18 patients had demyelinating neuropathy & the remaining (65) had mixed demyelinating & axonal neuropathy as shown in figure 4.4.

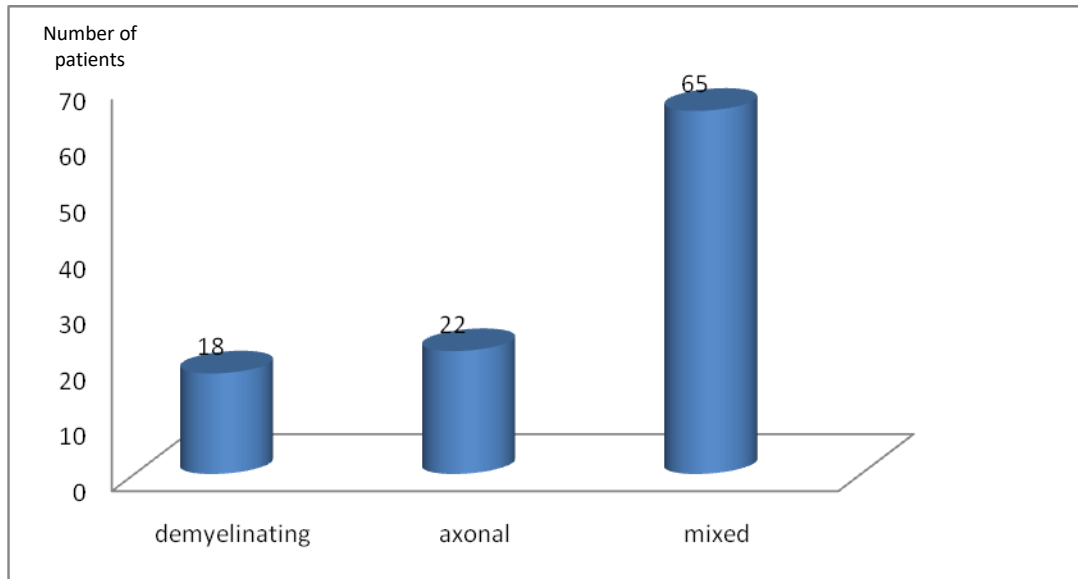


Figure 4.4 Types of diabetic peripheral neuropathy according to the pathophysiological process.

CHAPTER FIVE

Discussion

5.1 Age distribution:-

The results of the study show that there are statistically significant differences in age distribution between patients with diabetic peripheral neuropathy and diabetics without neuropathy (Table 4.1). These results agree with the results of other studies (Chang *et al.*, 2000; Kastenbauer, *et al.*, 2003; Sandberg and Wickblade, 2003; Booya *et al.*, 2005; Shiraiwa *et al.*, 2005; Kiziltan *et al.*, 2007 and Sorenson *et al.*, 2008) in that older age diabetics are at increased risk for developing DPN. While it does not agree with the results of other studies (Coppini *et al.*, 2001; Kawano *et al.*, 2001; Turgut, *et al.*, 2004; Costa *et al.*, 2006 and Uluc *et al.*, 2008) that do not show statistically significant differences in age between patients and control group. The explanation of these results is:- old age is associated with longer duration of diabetes, presence of other diseases or administration of drugs that might aggravate neuropathy (Damci *et al.*, 1999).

5.2 Gender differences:-

The study shows that males are more prone to develop neuropathic complications of DM than females (Table 4.2 and 4.3). This result agrees with results of other studies (Olsen *et al.*, 2000; Coppini *et al.*, 2001; Sorenson *et al.*, 2002; Booya *et al.*, 2005; Kiziltan *et al.*, 2007 and Cardoso and Salles, 2008) in that male gender is a risk factor for DPN. These results are related to that males are at higher risk to develop atherosclerotic disease than females that are predisposed to microvascular diseases like DPN.

Additionally, males have higher range of heights when compared to females (height is a known as a risk factor for DPN) (Shaw *et al.*, 1998).

5.3 Types of diabetes mellitus:-

The study shows higher prevalence of DPN in patients with Type 1 DM. This result agrees with results of other studies (Coppini *et al.*, 2001 and Harati, 2007) in that Type 1 DM is associated with higher incidence of DPN (figure 4.1). The explanation of this result is that Type 1 DM is associated with absolute deficiency of insulin and C-peptide. C-peptide has many insulin like and neuroprotective properties and so its deficiency will result in severer and rapid progression of DPN (Sima, 2004).

5.4 Duration of diabetes mellitus:-

The study shows that DPN patients have longer duration of diabetes when compared with diabetics without neuropathy (Table 4.4). The result of study here agrees with other studies (Kastenbauer, *et al.*, 2003; Sandberg and Wickblad, 2003; Brown *et al.*, 2004; Booya *et al.*, 2005; Shiraiwa *et al.*, 2005; Costa *et al.*, 2006; Kiziltan, *et al.*, 2007; Wintrob *et al.*, 2007; Cardoso and Salles, 2008 and Van Acher, *et al.*, 2009) in that longer duration of DM is a risk factor for the development of DPN. Kawano *et al.*, (2001) found out that there is a positive correlation between the prevalence of diabetic neuropathy and diabetes duration. Also, the results of the study shows a higher incidence of diabetic neuropathy in patients with duration of diabetes from 10 to 20 years when compared with those with duration of diabetes from 5-10 years and in patients with duration of diabetes from 5 to 10 years when compared to those with duration of diabetes below 5 years (figure 4.1) and so it agrees with a study done by Nather, *et al.*, (2008). These results is

assumed to be caused by:- longer duration of DM is associated with poorer control of DM, accumulation of injurious effects of poor metabolic control on peripheral nerves, severer atherosclerosis which results in microvascular insufficiency and accelerates neuropathy (Edward's *et al.*, 2008).

5.5 Clinical features of diabetic peripheral neuropathy:-

5.5.1 Symptoms of diabetic peripheral neuropathy:-

Parasthesia and numbness of distal parts of the limbs (fingers and toes) are the main presented symptom followed by pain of muscles or burning pain of distal extremities and weakness of proximal muscles (Table 4.5). These results agree with results of other studies (Kawano, *et al.*, 2001; Costa *et al.*, 2006; Kiziltan, *et al.*, 2007; Wintrob *et al.*, 2007; Aaberg *et al.*, 2008; Hyllienmark *et al.*, 2009). Moreover, the study agrees with Kastenbauer, *et al.*, (2003) who found that paresthesia and numbness were the most common complain of DPN followed by pain (burning) and weakness. Rubino *et al.*, (2007) found that numbness was the most commonly recorded DPN symptom in France, Italy, and Spain. This higher prevalence of paresthesia, numbness and pain are due to the fact that small sensory fibers affected early in the course of disease and these symptoms are due to small sensory fiber dysfunction. While motor weakness is due to large fiber dysfunction which occurs late in the disease (Kawano *et al.*, 2001).

5.5.2 Signs of diabetic peripheral neuropathy:-

Reduced vibration perception threshold of big toe and medial malleolus were the most common and early abnormalities found during neurological test, followed by reduced deep tendon reflexes and impaired pinprick sensation of distal parts of body (stock and glove sensory loss) and the last

abnormality was reduced power of upper or lower limbs (power grade 3 and 4) (Table 4.5). These results agree with results of other studies (Kawano *et al.*, 2001; Costa, *et al.*, 2006; Kiziltan *et al.*, 2007 and Wintrob *et al.*, 2007). Costa, *et al.*, (2006) found out that abnormal vibration perception threshold occur early in DPN than other signs and can be used as a useful screening tool for DPN in a symptomatic Type 2 diabetics. Olsen *et al.*, (2000); Coppini *et al.*, (2001) and Kamei *et al.*, (2005) use toe vibration thresholds as a marker for follow up of diabetic neuropathy and it is shown that it is a useful predictor of lower extremity complications. Moreover, Coppini *et al.*, (2001) found that although clinical tests (cotton wool sensation, ankle reflexes) were normal in most patients (90%) at baseline, vibration sensation were significantly abnormal in patients who developed DPN. The high sensitivity of vibration perception in the detection of neuropathy is due to the vibrating stimuli, as opposed to simple mechanical pressure, requires high-fidelity afferent conduction and is thus sensitive to be lost by distal axonopathy (vibration perception assesses the function of the Meissner and Pacinian corpuscles and their associated large-diameter fibers)(Manivannan *et al.*, 2008). While, Uluc *et al.*, (2008) found that reduced pinprick sensitivity and temperature loss were the most common abnormal neurological signs followed by reduced vibration sensation and abnormal proprioception. Reduced muscle stretch reflexes at the ankles were the least common neurological sign. This study shows that abnormalities of sensory system starts early during the course of disease because sensory fibers are affected earlier than motor fibers. Also, the results of this study indicate that the signs and symptoms of DPN start in the lower limbs because peripheral nerves in the lower limbs are the longest in the body and are more prone to

injuries (demyelinating and others) that are predisposed to DPN (Weisswasser *et al.*, 2003). Also the results of the study show that the signs and symptoms of DPN are bilateral because the pathological process of DPN is systemic and involves all the nerves simultaneously (Nather *et al.*, 2008).

5.6 Fasting blood sugar:-

The study shows higher fasting blood sugar in DPN patients than control groups with statistically significant difference (Table 4.7). The study agrees with previous studies (Chang *et al.*, 2000; Coppini, *et al.*, 2001; Sandberg and wickblad, 2003; Booya, *et al.*, 2005 and Cardoso and Salles 2008) in that hyperglycemia is the most important risk factor for DPN. Chronic hyperglycemia represents the main causative factor involved in the pathogenesis of diabetic neuropathy. Nerve damage may be directly induced by the accumulation of intracellular glucose (the consequences of which include the generation of glycating sugars and advanced glycation end-products (AGE), enhanced oxidative damage and protein kinase C activation). Apart from direct hyperglycemia-induced damage, ischemia caused indirectly from decrease in neurovascular flow almost certainly plays a role (Boucek, 2006).

5.7 Glycosylated Hemoglobin (HbA1c):-

The results of HbA1c of DPN patients are higher than those of control groups with statistically significant differences (Table 4.7). These results agree with results of other studies (Coppini, *et al.*, 2001; Sandberg and wickblad, 2003; Booya *et al.*, 2005; Shiraiwa *et al.*, 2005 and Wintrob *et al.*, 2007) in that higher HbA1c values is reported in DPN patients. High HbA1c values indicate elevated mean blood sugar value over the last 2 months.

5.8 Lipid profile abnormalities:-

5.8.1 Total cholesterol (TC):-

Total cholesterol values of DPN patients are higher than those of control groups without statistically significant differences (Table 4.8). These results agree with results of other studies (Maser *et al.*, 1996; Rajmohan *et al.*, 2000; Fried *et al.*, 2001; Booya *et al.*, 2005; Shiraiwa *et al.*, 2005; Agrawal *et al.*, 2006 and Costa *et al.*, 2006) in that total cholesterol level is not a risk factor of DPN.

5.8.2 Triglycerides (TG):-

Triglyceride values of DPN patients are higher than those of control groups without statistically significant differences (Table 4.8). These results agree with results of previous studies (Maser *et al.*, 1996; Takihashi *et al.*, 1998; Fried *et al.*, 2001; Booya *et al.*, 2005; Shiraiwa *et al.*, 2005 and Agrawal *et al.*, 2006) in that TG level is not a risk factor of DPN. While Costa *et al.*, (2006) and Van Acker *et al.*, (2009) show that DPN patients have higher TG level than control.

5.8.3 Very Low Density Lipoprotein (VLDL):-

The VLDL values of DPN patients are higher than those of control without statistically significant differences (Table 4.8). These results agree with results of previous studies (Maser *et al.*, 1996; Booya *et al.*, 2005; Shiraiwa *et al.*, 2005 and Agrawal *et al.*, 2006) in that VLDL level is not a risk factor of DPN.

5.8.4 High Density Lipoprotein (HDL):-

HDL values of DPN patients are lower than those of control without statistically significant differences (Table 4.8). this result agree with results like Maser *et al.*, (1996); Takihashi *et al.*, (1998); Fried *et al.*, (2001); Booya

et al., (2005) and Agrawal *et al.*, (2006) that do not show significant differences in HDL level between patients and control.

These results do not agree with results of Costa *et al.*, (2006); Cardoso and Salles, (2008) and Van Acker *et al.*, (2009) who found out that lower serum HDL cholesterol was a risk factor of DPN and it was a protective factor for neuropathy and this probably reflects the interactions with the presence of peripheral arterial disease in determining progression of peripheral neuropathy, since a low HDL cholesterol is a well-known risk factor for diffuse atherosclerotic disease.

5.8.5 Low Density Lipoprotein (LDL):-

The LDL values of DPN patients are higher than those of control without statistically significant differences (Table 4.8). These results agree with results of previous studies (Maser *et al.*, 1996; Takihashi *et al.*, 1998; Fried *et al.*, 2001; Booya *et al.*, 2005; Shiraiwa *et al.*, 2005 and Agrawal *et al.*, 2006) in that LDL level is not a risk factor of DPN.

The cause of results of lipid profile (TC, HDL, LDL, VLDL and TG) of the study is that those patients had advanced disease with long duration of DM, so they have a diet regimen that excludes many food types that contain saturated fatty acids and cholesterol (Fried *et al.*, 2001).

5.9 Serum selenium level:-

The study shows that selenium level is statistically lower in DPN patients than control groups (Table 4.9). Selenium is a trace element with powerful antioxidant effect and the finding of low level of selenium in DPN patients is assumed to the fact that DM is associated with enhanced oxidative stress which causes depletion of these antioxidants. When the level of selenium and other antioxidants drop, it will reduce the antioxidant defense

mechanisms of the body and enhance oxidative stress (Pedrero and Madrid, 2009). Enhancement of oxidative stress is a well known pathological pathway in the pathogenesis of DPN (Driskell *et al.*, 2006).

5.10 Serum vitamin E level:-

The study shows that levels of vitamin-E were lower in patients with DPN than control groups with statistically significant differences (Table 4.9). These results agree with result of previous studies (Van Dam *et al.*, 1998; Alazawie and Alhamdani, 2006; Singh, *et al.*, 2008).

The finding of low level of vitamin E in DPN patients is due to the fact that DM is associated with enhanced oxidative stress which causes depletion of these antioxidants. Also vitamin E is a fat soluble vitamin and patients with DM are likely to be on diet regimen that excludes fat and so they are more prone to develop vitamin E deficiency (Van Dam *et al.*, 1998). When the level of Vitamin E drops, it will reduce the antioxidant defense mechanisms of the body and enhance oxidative stress. Enhancement of oxidative stress is a well known pathological pathway in the pathogenesis of DPN (Tinggi, 2003). Willems *et al.*, (1998) found that the level of vitamin E was normal in young Type 1 diabetics with good glycemic control indicating the importance of hyperglycemia in causing deficiency in antioxidants.

5.11 Electrophysiological assessment:-

5.11.1 Median nerve:-

a- Results of motor nerve conduction study

The study shows that there are significant differences in motor latency and conduction velocity between patients and control groups (Table 4.10). These

results agree with results of other studies (Uncini, *et al.*, 1999; Karsidag *et al.*, 2005 and Ali, 2007).

b- Results of sensory nerve conduction study.

The study shows that there are significant differences in sensory latency and amplitude between patients and control groups (4.11). These results agree with results of other studies (Karsidag *et al.*, 2005 and Ali, 2007).

5.11.2 Ulnar nerve:-

a- Motor nerve conduction study:-

There are significant differences regarding motor latency, amplitude and conduction velocity between patients and control groups (4.12). These results agree with results of other studies (De Wytt *et al.*, 1999; Uncini, *et al.*, 1999; Karsidag *et al.*, 2005; Ali, 2007 and Al-Muhanna, 2008).

b- Sensory nerve conduction study:-

There are significant differences regarding sensory latency between patients and control groups (4.13). These results agree with results of other studies (De Wytt *et al.*, 1999; Karsidag *et al.*, 2005; Ali, 2007 and Al-Muhanna, 2008).

5.11.3 Common peroneal nerve: -

There are significant differences regarding conduction velocity between patients and control groups (Table 4.14). These results agree with results of other studies (Takahashi *et al.*, 1998; Uncini, *et al.*, 1999; Guttierrez *et al.*, 2001; Karsidag *et al.*, 2005; Ali, 2007).

5.11.4 Posterior tibial nerve: -

There are significant differences regarding motor latency, amplitude and conduction velocity between patients and control groups (Table 4.15). These results agree with results of other studies (De Wytt *et al.*, 1999; Karsidag *et al.*, 2005 and Ali, 2007).

5.11.5 Sural nerve: -

There are significant differences regarding sensory latency between patients and control groups (Table 4.16). These results agree with results of other studies (Takihashi *et al.*, 1998; Guttierrez *et al.*, 2001; Turgut *et al.*, 2004; Karsidag *et al.*, 2005 and Ali, 2007).

The results of nerve conduction study of all nerves (motor nerves that includes:- median, ulnar, common peroneal, posterior tibial and sensory nerves that includes:- median, ulnar and sural) are due to electrophysiological changes of diabetic neuropathy are due to affection of large myelinated fast conducting fibers (small unmyelinated fibers are not tested by routine electrophysiological techniques). Large fiber dysfunction causes abnormalities in vibration perception and conduction velocity (Dyck and Thomas, 2005). Early patients with DPN might remain asymptomatic and discovered by routine examination or nerve conduction study. The earliest changes are:- development of mild reduction in conduction velocity and amplitude and most of these changes are recognized at the distal parts of lower limbs and at sensory nerves with normal motor nerves (at this stage it is referred to as sensory neuropathy). Later on there will be much reduction in conduction velocity and amplitude, involvement of both sensory and motor fibers (mixed sensory and motor neuropathy) and the changes ascends up to involve proximal parts of lower limbs and upper limbs. Finally patients

may develop severe neuropathy with prominent clinical features and nerve conduction abnormalities that might lead to no response of one or more than one nerve on examination and nerve conduction study. In addition to that, results of study shows that most of patients (62%) developed mixed demyelinating and axonal neuropathy, while the remainder develops either pure axonal or demyelinating neuropathy. The pathophysiology of DPN is demyelination and axonal degeneration (Kimura, 2001). The demyelinating neuropathy is due to demyelination injuries. Demyelination injuries are segmental along the nerve fiber and cause progressive deterioration in nerve function and nerve conduction study parameters. Demyelinating neuropathy causes much prolongation in distal latency and decrease in conduction velocity (>30-40% reduction) with little effect on amplitude. Axonal degeneration injuries affects primarily the axon and characterized by dying back degeneration of distal portions of axon causes much reduction in amplitude with little reduction in conduction velocity (<20-30% reduction). This can be explained by the fact that since the amplitude of the compound muscle action potential (CMAP) is a function of the number and size of nerve and muscle fibers “ the number of the functioning axons in a nerve, and the amount of muscle still innervated “ (and for sensory nerve action potential is a function of the number of the functioning axons in the nerve) (Daube, 1999), so it is believed that in axonal degeneration there are less functioning axons in the nerve fiber which reduces the amplitude. While conduction velocity is a function of fiber size, myelination, nodal and internodal length and external and internal axonal resistance, so demyelination (means loss of myelin sheath which is responsible for fast

conduction in large fibers) will cause much reduction in conduction velocity than amplitude (Dyck and Thomas, 2005 and Al-Muhana, 2008).

5.12 Nerves involvement:-

The study shows that not all nerves involved at the same time and so the patient may have 2 or more than 2 nerve involved (the diagnosis of DPN is based on the presence of 2 or more than 2 nerves affected) according to the severity of the pathological process (figure 4.3). The study shows that the most common nerve involved by DPN is sural nerve followed by median sensory nerve, common peroneal nerve, posterior tibial nerve, ulnar sensory nerve, median and ulnar motor nerves and so it agree with results of Karsidag *et al.*, (2005).

These results shown to be due to the fact that pathological process of DPN starts in the longest nerves of the body and in its distal ends (what is referred to as dying back degeneration) and so the clinical features and electrophysiological changes of DPN start at the nerves of the lower limbs and then ascends up as the disease advances (Karsidag, *et al.*, 2005). Also the study shows higher prevalence of median sensory nerve dysfunction indicating the higher prevalence of carpal tunnel syndrome (stamboulis *et al.*, 2009). Moreover the study shows that the nerve involvement is symmetrical because the pathological process is generalized and affects all nerves of the body simultaneously. Furthermore, the study reveals that sensory system is affected more than and earlier than motor system because sensory fibers are affected earlier (Aminoff, 2005).

Conclusions

- 1- The clinical study of patients with diabetic peripheral neuropathy shows that paresthesia and numbness are the main presenting symptoms, while decrease vibration perception threshold and deep tendon reflexes respectively are the most common and early signs of DPN.
- 2- Duration of DM, type 1 diabetes and male gender are important risk factors of DPN.
- 3- The study shows significantly higher value of fasting blood sugar and Glycosylated hemoglobin in DPN patients than control. Nevertheless, it shows non significant increase in lipid parameters (TC, TG, LDL and VLDL) and non significant decrease in HDL in DPN patients than control which might be due to restriction of dietary stuffs rich in fat, in addition to that, the study of selenium and vitamin E shows significant decrease in these antioxidant parameters indicating that there is a role of oxidative stress in the pathogenesis of DPN.
- 4- The electrophysiological study shows the following:- a- the pathophysiological pattern of DPN in the form of demyelination and axonal degeneration. B- Symmetrical involvement of peripheral nerves. C- Polyneuropathy start early in the nerves of lower limbs since these are the longest nerves in the body.

Recommendations

- 1- Screening for diabetic peripheral neuropathy in outpatient clinics and hospitals by simple procedures including history and examination (specifically vibration perception threshold) is important. This helps in early diagnosis and early institution of therapy. The screening should focus on patients with longer duration of diabetes because they are more liable to develop DPN.
- 2- Control of blood sugar level is the most important factor in the prevention of DPN.
- 3- Diabetic patients should be encouraged to eat foodstuffs rich with antioxidants like vegetables, garlic oil, fish and others.
- 4- Electrophysiological study should be done for every diabetic patient to diagnose subclinical diabetic polyneuropathy and so help for early institution of therapy. The Electrophysiological study should focus on lower limbs because DPN start early in lower limbs.

List of abbreviations

AGE	Advanced glycation end product
ARI	Aldose reductase inhibitors
CMAP	Compound muscle action potential
CV	Conduction velocity
DM	Diabetes Mellitus
DML	Distal motor latency
DPN	Diabetic peripheral neuropathy
DSL	Distal sensory latency
FBS	Fasting blood sugar
GSH	Glutathione
HbA1c	Glycosylated haemoglobin A1c
HDL	High density lipoprotein
LDL	Low density lipoprotein
mA	milliamper
mg/dl	milligram/decilitre
mmol/l	mill mol/litter
MRI	Magnetic resonance image
NCS	Nerve conduction study
NCV	Nerve conduction velocity
nm	Nanometer
PNS	Peripheral nervous system
ROS	Reactive oxygen species
SNAP	Sensory nerve action potential
SS	Serum selenium
VLDL	Very low density lipoprotein
MFCV	Muscle fiber conduction velocity
IGF	Insulin like growth factor
DRG	Dorsal root ganglia

Abstract

This study was designated to estimate some clinical, biochemical and electrophysiological changes in patients with diabetic peripheral neuropathy, since the changes in these parameters are important in detecting, quantifying, identifying some possible risk factors and assessing patient's response to treatment.

The study lasted from November/2008 to May/2009. The total number of study group was 225 persons who fell into 105 patients and 120 controls. The patients were classified into two groups according to the type of DM into: patients with type 1 DM (35 patients), their age range between 16-34 years and patients with type 2 DM (70 patients), their age range between 45-72 years. While control group was classified into diabetic patients without neuropathy (60 patients), their age ranged between 18-52 years and normal healthy control (60 persons), their age ranged between 18-70 years. The patients were recorded in Marjan's teaching hospital center of diabetes and had monthly visits. The patients and control were examined by specialist doctors.

The patients and controls enrolled in the study had undergone full assessment that included:- clinical assessment (history and full examination), biochemical assessment (measurement of fasting blood sugar, lipid profile, serum selenium and vitamin E), measurement of glycosylated hemoglobin and electrophysiological assessment that included sensory and motor nerve conduction studies.

The study revealed that parasthesia and numbness were the most common symptoms of diabetic polyneuropathy followed by pain (cramping or burning pain) and muscle weakness. While the most common and early sign found during neurological examination was impaired vibration perception

threshold of big toe and medial malleolus followed by diminished deep tendon reflexes of lower limbs joints, then impaired pinprick and thermal sensitivity and finally there was muscle weakness. As well as the study showed that fasting blood sugar and HbA1c level were higher in patients with neuropathy than controls with statistically significant differences ($p < 0.05$). The lipid profile parameters were higher in patients than controls without significant differences ($p > 0.05$). While serum selenium and vitamin E level show significant decrease ($p < 0.05$) in patients with diabetic peripheral neuropathy than controls.

Regarding electrophysiological changes patients had shown significant increase in latency for sensory and motor nerves and significant decrease in amplitude and conduction velocity for sensory and motor nerves in patients with peripheral neuropathy than controls.

The study found out that hyperglycemia due to deficiency of insulin or resistance to its metabolic effect is the most important and correctable risk factor for peripheral neuropathy, in addition to the role of hyperglycemia, oxidative stress also plays role.

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References

- Aaberg, M.L.; Burch, D.M.; Hud, Z.R. and Zacharias, M.P. (2008). Gender differences in the onset of diabetic neuropathy. *Journal of Diabetes and Its Complications*. 22:83– 87.
- Agrawal,R. P.; Sharma, P.; Pal, M.; Kochar, A. & Kochar, D. K. (2006). Magnitude of dyslipedemia and its association with micro and macro vascular complications in type 2 diabetes: A hospital based study from Bikaner (Northwest India). *Diabetes Research and Clinical Practice*, 73:211–214.
- Ahmed, N. (2005). Advanced glycation endproducts—role in pathology of diabetic complications. *Diabetes Research and Clinical Practice*, 67:3–21.
- Akbar, D.H.; Mira, S.A.; Zawawi, T.H. & Malibary, H.M. (2000). Subclinical diabetic neruopathy, a common complication in Saudi diabetics. *Saudi Med J*; 21:433–7.
- Al-Azzawie, H.A. & Alhamdani, M.S. (2006). Hypoglycemic and antioxidant effect of oleuropein in alloxan-diabetic rabbits. *Life Sciences*, 78:1371 – 1377.
- Ali, S. H. (2007). Clinical, electrophysiological & biochemical assessment of various chronic acquired demyelinating polyneuropathy phenotypes. College of Medicine Al-Mustansiryah university. (Ph.D thesis).
- Al-Muhanna, M.Y.I. (2008). An electrophysiological study to the effect of changing body posture on the peripheral nerves in healthy

- subjects & diabetic patients. College of medicine. Al-Kufa university. (M.Sc thesis).
- Aminoff, M.J. (1999). *Electrodiagnosis in clinical neurology*, fourth edition. New York: Churchill Livingstone.
- Anderioli, T. E.; Carpenter, C. C.; Griggs, R. C. & Loscalzo, J. (2004). *Cecil's essentials of medicine*. 6th ed. Saunders. USA, 392-399.
- Arezzo, J. C. (1999). New Developments in the Diagnosis of Diabetic Neuropathy. *Am J Med.*, 107(2B):9S–16S.
- Baker, H. & Frank, O. (1968). *Clinical vitaminology*. New York: Wiley. 172.
- Booya, F.; Bandarian, F.; Larijani, B.; Pajouhi, M.; Nooraei, M. & Lotfi, J. (2005). Potential risk factors for diabetic neuropathy: a case control study. *BMC neurology*, 5:24.
- Bostock, H.; and Grafe, P. (1985). Activity–dependent excitability changes in normal and demyelinated rat spinal root axons. *J. Physiol (London)*.; 365: 239-257.
- Boucek, 2006. Advanced Diabetic Neuropathy: A Point of no Return? *The Review of Diabetic Studies*, 3(3):144-152.
- Boulton, A. J.; Vinik, A. I.; Arezzo, J. C.; Bril, V.; Feldman, E. L. and Freeman, R. (2005). Diabetic neuropathies: a statement by the American Diabetes Association. *Diabetes Care*, 28(4), 956–962.
- Bragd, J.; Adamson, U.; Bäcklund, L.B.; Lins, P.E.; Moberg, E. & Oskarsson, P. (2008). Can glycaemic variability, as calculated

from blood glucose self-monitoring, predict the development of complications in type 1 diabetes over a decade? *Diabetes & Metabolism*, 30:33–37.

Brown, L.M.; Cunningham, J.; Dunne, K.; Whitbread, C.; Howard, D.; Weeramanthri, T.; Tatipata, S.; Dunbar, T.; Harper, C.A.; Taylor, H.R.; Zimmet, P.; O'Dea, K. and Shaw, J.E. (2008). Complications of diabetes in urban Indigenous Australians: The DRUID study. *Diabetes research and clinical practice*. 80:455–462.

Burits, C. A. & Ashwood, E. R. (1999). *Teitit textbook of clinical chemistry*. 4th. Ed. W. B., Saunders comp. USA, 2 (1500-1503).

Burke, D.; Kiernan, M.C. & Bostock, H. (2001). Excitability of human axons. *Clin Neurophysiol*, 112:1575–85.

Campbell, S.C.; Aldibbiat, A.; Marriott, C.E.; Landya, C.; Ali, T.; Ferris, W.; Butler, C.S.; Shawb, J.A. & Macfarlane, W.E. (2008). Selenium stimulates pancreatic beta-cell gene expression and enhances islet function. *FEBS Letters*, 582:2333–2337.

Cardoso, K. R. L. & Salles, L. f. (2008). Predictors of development & progression of microvascular complications in a cohort of Brazilian type 2 diabetic patients. *Journal of diabetes & its complications*, 22:164-170.

Chang, C.; Lu, F.; Yang, Y.; Wu, J.; Wu, T.; Chen, M.; Chuang, L. & Tai, T. (2000). Epidemiologic study of type 2 diabetes in Taiwan. *Diabetes Research and Clinical Practice* 50 Suppl. 2:49–59.

- Coppini, D.V.; Wellmer, A.; Weng, C.; Young, P.J.; and, Sönksen, H. (2001). The natural history of diabetic peripheral neuropathy determined by a 12 year prospective study using vibration perception thresholds *Journal of Clinical Neuroscience*. 8:520–524.
- Costa, A.L; Maraschin, A. F; Decastro, J. H. X.; Grosso, J.L & Friedman, R. (2006). A simplified protocol to screen for distal polyneuropathy in type 2 diabetic patients. *Diabetes Research and Clinical Practice*, 73:292–297
- Damci, T.; Osar, Z.; Beyhan, S.; Ilkova, H.; Ozyazar, M.; Gorpe, U. & Bagriacik, N. (1999). Does instantaneous blood glucose affect vibration perception threshold measurement using biothesiometer?. *Diabetes Research and Clinical Practice* 46, 19–22.
- Daniel, W. W. (1999). *Biostatistic: A foundation for analysis in health science*. 7th ed. John Wiley. Philadilphia, p 55.
- Daube, J.R. (1999). Electrodiagnostic testing in diabetic neuropathy. In Dyck, PJ and Thomas, PK (eds): *Diabetic Neuropathy* 2nd ed. W.B. Saunders co. Philadelphia pp: 222-238.
- David, R. C. (2001). Diagnosis and management of peripheral nerve disorders. *Electrodiagnostic Evaluation of the peripheral neuropathy*. 30-37.
- Davidsons, (2006). *Principles & practice of medicine*. 18th edition. Churchil Livingston: 420-520.

- De Wyt, C.N.; Jackson, R.V.; Hockings, G.R.; Joyner, J.M. & Strakosch, C.R. (1999). Polyneuropathy in Australian Outpatients with Type II Diabetes Mellitus. *Journal of Diabetes and Its Complications*. 13:74–78.
- Dobertsov, M.; Romanovesky, D. & Stimers, J. R. (2007). Early diabetic neuropathy: Triggers and mechanisms. *World J Gastroenterol*, 13(2): 175–191.
- Driskell, J.A.; Giraud, D.W.; Drewel, B.T. & Davy, S.R. (2006). Dietary intakes and plasma concentrations of vitamin C, selenium, and carotenoids of a group of preschool children. *Nutrition Research*, 26:23– 26.
- Dyck, P. J. & Thomas, P.K. (2005). *Diabetic neuropathy*. 2nd edition. W.B. Saunders, Philadelphia. 100-420.
- Dyck, P.J.; Davies, J.L. and Wilson, D.M. (1999). Risk factors for severity of diabetic polyneuropathy Intensive longitudinal assessment of the Rochester Diabetic Neuropathy Study cohort. *Diabetes Care*, 22(9):1479–86.
- Dyck, P.J.; Davies, J.L. and Wilson, D.M. (1999). Risk factors for severity of diabetic polyneuropathy. Intensive longitudinal assessment of the Rochester Diabetic Neuropathy Study cohort. *Diabetes Care.*, 22:1479–86.
- Edward's, J. L.; Vincent, A. M.; Cheng, H. T. & Feldman, E. L. (2008). *Diabetic neuropathy: mechanisms to management*. *Pharmacology & therapeutics*, 120:1-34.

- Eliasson, B. (2003). Cigarette smoking & diabetes. *Progress in Cardiovascular Diseases*, 45:405-413.
- Frank P (1988). Monitoring the diabetic patient with glycosylated hemoglobin measurements. *Clin. Prod.*, 4:112.
- Frieda, L.F.; Kimberly, Y.Z.; Ellis, D.; Changb, Y.; Silvers, N. & Orchard, T.J. (2001). Lipid modulation in insulin-dependent diabetes mellitus effect on microvascular outcomes. *Journal of Diabetes and Its Complications*, 15:113–119.
- Fujita, Y.; Fukushima, M.; Suzuki, H.; Taniguchi, A.; Nakai, Y.; Kuroe, A.; Yasuda, K.; Hosokawa, M.; Yamada, Y.; Inagaki, N. & Seino, Y. (2008). Short term intensive glycemic control improve vibratory sensation in type 2 diabetes. *Diabetes research & clinical practice*, 80:160-190.
- Ganong, W.F. (2005). *Physiology of the nerve and muscle cells Review of Medical Physiology*, 16th ed., 37-97.
- Gin, H.; Perlemoine, c. & Rigalleau, v. (2006). How to better systematize the diagnosis of neuropathy? *Diabetes Metab.*, 32:367-372.
- Girach, A. and Vignati L. (2006). Diabetic microvascular complications—can the presence of one predict the development of another? *Journal of Diabetes and Its Complications*, 20:228– 237.
- Goldstein, D.E. (1999). Is glycosylated hemoglobin clinically useful?. *N Eng J Med*, 310-364.

- Greenberg, A. (2004). EMG pearls, section 2, Generalized Neuropathies. 94-172.
- Gutierrez, E.M.; Helber, M.D.; Dealva, D.; Ashton-Miller, J.A. & Richardson, K.A. (2001). Mild diabetic neuropathy affects ankle motor function. *Clinical biomechanics*, 16:522-528.
- Guyton, A. C. & Hall, J. E. (2006). Textbook of medical physiology, 11th edition. By WB Sanders company: 972-977.
- Harati, Y. (2007). Diabetic Neuropathies: Unanswered Questions. *Neurol Clin*, 25:303–317.
- Harrison, S. (2002). Harrison's Principles of Internal Medicine, 14th ed. New York, Mc-Graw-Hill. 2372-2378.
- Hashim, S.A. & Schutringer, G.R. (1966). Rapid determination of tocopherol in macro & microquantities of plasma results obtained in various nutrition & metabolic studies. *Am.J.Clin. Nut.*, 19 (2):137-145.
- Hyllienmark, L.; Golster, H.; Samuelsson, U. & Ludvigsson, J. (2001). Nerve conduction defects are retarded by tight metabolic control in type 1 diabetes. *Muscle Nerve*; 24:240–6.
- Imada, M.; Misawa, S.; Sawai, S.; Tamura, N.; Kanai, K., Sakurai, K.; Sakamoto, S.; Nomura, F.; Hattori, F. and Kuwabara, S. (2007). Median-radial sensory nerve comparative studies in the detection of median neuropathy at the wrist in diabetic patients. *Clinical Neurophysiology*, 118:1405–1409.
- Kamei, N.; Yamane, K.; Nakanishi, S.; Yamashita, Y. & Tamura, T. (2003). Effectiveness of Semmes–Weinstein monofilament

examination for diabetic peripheral neuropathy screening. *Journal of Diabetes and Its Complications*, 19:47– 53.

Karsidaga, S.; Morali, S.; Sargin, M.; Salman, S.; Karsidag, K. & Usa, O. (2005). The electrophysiological findings of subclinical neuropathy in patients with recently diagnosed type 1 diabetes mellitus. *Diabetes Research and Clinical Practice*, 67:211–219.

Kastenbauer, T; Irsigler, P; Sausenga, S.; Grimmer, A. & Prager, R. (2004). The prevalence of symptoms of sensorimotor and autonomic neuropathy in Type 1 and Type 2 diabetic subjects. *Journal of Diabetes and Its Complications*, 18:27– 31.

Kawano, M.; Omori, Y.; Katayama, S.; Masanobu Kawakami, M.; Suzuki, Y.; Takahashi, K.; Takemura, Y.; Nagata, N.; Hiratsuka, A.; Matsuzaki, F.; Kanazawa, Y. and Akanuma, Y. (2001). A questionnaire for neurological symptoms in patients with diabetes — cross-sectional multicenter study in Saitama Prefecture, Japan. *Diabetes Research and Clinical Practice*, 54 :41–47.

Kikkawa, Y.; Kuwabara, S.; Misawa, S.; Tamura, N.; Kitano, Y.; Ogawara, K. & Hattoria (2005). The acute effects of glycemic control on nerve conduction in human diabetics. *Clinical Neurophysiology*, 116:270–274.

Killian, J.M. & Foreman, P.J. (2001). Clinical utility of dorsal sural nerve conduction studies. *Muscle Nerve*, 24:817–20.

Kimura, J. (2001) *Electrodiagnosis in Diseases of Nerve and Muscle: Principles and Practice*, 3rd ed. Oxford University Press.

- Kiziltan, M.E.; Gunduz, A.; Kiziltan, G.; Akalin, M.A. & Uzun, N. (2007). Peripheral neuropathy in patients with diabetic foot ulcers: Clinical and nerve conduction study. *Journal of the Neurological Sciences*, 258:75–79.
- Kumar, A.; Kaundal, R.K.; Iyer, S. & Shyam, S. (2007). Effects of resveratrol on nerve functions, oxidative stress and DNA fragmentation in experimental diabetic neuropathy *Sharma Life Sciences* 80:1236–1244.
- Kuwabara, S. (2003). Shortened refractory periods in human diabetic neuropathy. *Clinical Neurophysiology*, 114:169–170.
- Leiter, L. A. (2005). The prevention of diabetic microvascular complications of diabetes: Is there a role for lipid lowering? *Diabetes Research and Clinical Practice*, 68S2:S3–S14
- Lewis, S. M.; Bain, B. J. & Bates, I. (2006). *Dacei & Lewis practical hematology*. 10th ed., Churchill Livingstone Elsevier. Germany.
- Leyden, E.; Beitrag, zur. Klinik des Diabetes mellitus. *Wien Med Wochenschr* 1893;43:926, cited by Tracy & Dyck, 2008.
- Manivannana, M.; Periyasamy, R. & Narayanamurthyb, B. (2008). Vibration perception threshold and the law of mobility in diabetic mellitus patients. *Primary care diabetes*, 30:30–33.
- Maser, R.E.; Usher, DE. & DeCherney, G.S. (1996). Little Association of Lipid Parameters and Large Sensory Nerve Function in Diabetes Mellitus. *Journal of Diabetes and Its Complications*, 54-49.

- Meijera, J.W.G.; Lange, F.; Linksd, T.P. & van der Hoeven, J.H. (2008) Muscle fiber conduction abnormalities in early diabetic polyneuropathy. *Clinical Neurophysiology*, 119:1379–1384
- Misawa, S.; Kuwabara, S.; Ogawara, K.; Kitano, Y. & Hattori, E. (2004). Strength-duration properties & glycemic control in human diabetic motor nerves. *Clinical neurophysiology*, 116:245-258.
- Misawa, S.; Kuwabara, S.; Ogawara, K.; Kitano, Y.; Yagui, K. & Hattori, T. (2004). Hyperglycemia alters refractory periods in human diabetic neuropathy. *Clinical Neurophysiology* 115:2525–2529.
- Mrad, F.E.B.; Goulder, R.; Fredj, M.; Becher, S.B.; Mrad-Mazigh, S. & Mrad, A. (2000). Childhood diabetic neuropathy: a clinical and electrophysiological study. *Funct Neurol.*, 15:35–40.
- Mundet, X.; Pou, A.; Piquer, N.; Sanmartin, M.I.F.; Tarruella, M.; Gimbert, R. and Farrus, M. (2008). Prevalence and incidence of chronic complications and mortality in a cohort of type 2 diabetic patients in Spain. *Primary Care Diabetes*. 2:135-140.
- Nathan, D.M.; Singer, D.E.; Hurxthal, K. & Goodson, J.D., (1994). The clinical information value of the glycosylated hemoglobin assay. *Eng.J.Med.*, 310-345.
- Nather, A.; Neo, S.H.; Chionh, S.B.; Liew, S.C.F.; Sim, L.Y. and Chew, J.L.L. (2008). Assessment of sensory neuropathy in diabetic patients without diabetic foot problems. *Journal of Diabetes and Its Complications*, 22:126– 131.

- Obrosova, I. J. (2008). Diabetes & the peripheral nerve. *Biochimica et Biophysica Acta*, 30:30-40.
- Olsena, B.S., Sjølie, A.; Hougaard, P.; Johannesen, J.; Borch-Johnsen, K.; Marinelli, K.; Thorsteinsson, B.; Pramming, S. & Mortensen, H.B. (2000). A 6-year nationwide cohort study of glycaemic control in young people with Type 1 diabetes. Risk markers for the development of retinopathy, nephropathy, and neuropathy. Danish Study Group of Diabetes in Childhood *Journal of Diabetes and Its Complications*, 14:295– 300.
- Ozmen, B. & Boyvada, S. (2003). The relationship between self-monitoring of blood glucose control and glycosylated haemoglobin in patients with type 2 diabetes with and without diabetic retinopathy. *Journal of Diabetes and Its Complications*, 17:128–134.
- Park, T.S.; Park, J.H. and Baek, H.S. (2004). Can diabetic neuropathy be prevented? *Diabetes Research and Clinical Practice*, 66:53–56.
- Pedrero, Z. & Madrid, Y. (2009). Novel approaches for selenium speciation in foodstuffs and biological specimens: A review. *Analytica Chimica Acta*, 634:135–152
- Perkins, B.A.; Greene, D.A. & Bril, V. (2001). Glycemic control is related to the morphological severity of diabetic peripheral sensorimotor polyneuropathy. *Diabetes Care*;24:748–52.
- Rajmohan, L.; Deepa, R.; Mohan, A. & Mohan, V. (2000). Association between isolated hypercholesterolemia, isolated hypertriglyceridemia and coronary artery disease in South Indian type 2 diabetic patients. *Indian Heart J.*, 52:400–406.

- Rubino, A.; Rousculp, M.D.; Davis, K.; Wang, J.; Bastyr, E.J. and Tesfaye, S. (2007). Diagnosis of diabetic peripheral neuropathy among patients with type 1 and type 2 diabetes in France, Italy, Spain, and the United Kingdom. *primary care diabetes*, 1:129–134.
- Sandberg, G.E. & Wikblade, K.F. (2003). Oral dryness & peripheral neuropathy in subjects with type 2 DM. *Journal of Diabetes and Its Complications*, 17:192–198.
- Sandhu, J.S.; Aggrawal, S.P. & Narang, A.P.S. (1999). Lipid peroxidation in diabetic nephropathy. *Indian J Nephrology*. 3:97—9.
- Schermeister, J. (1964). The principles of estimation of creatinine. *Dtsch. Med. Wschr.*, 89: 1018, 1640.
- Schillaci, G.; Verdecchia, P.; Porcellati, C.; Cuccurullo, O.; Cosco, C. & Perticone, F. (2000). Continuous relation between left ventricular mass and cardiovascular risk in essential hypertension. *Hypertension*, 35:580– 586.
- Shaw, J.E.; Hodge, A.E.; De Courten, M.; Dowse, G.K.; Gareeboo, H.; Tuomilehto, J.; Alberti, K.G.M. & Zimmet, P.Z. (1998). *Diabetes Research and Clinical Practice*, 42:131–139.
- Shimoshige, Y.; Minoura, K.; Matsuoka, N.; Takakura, S.; Mutoh, S.; & Kamijo, M. (2009). Thirteen month inhibition of aldose reductase by zenarestat prevents morphological abnormalities in the dorsal root ganglia of streptozotocin induced diabetic rats. *Brain research*, 1247:182-187.

- Shiraiwa, T.; Kaneto, H.; Miyatsuka, T.; Kato, K.; Yamamoto, K.; Kawashima, A.; Kanda, T.; Suzuki, M.; Imano, E.; Matsuhisa, M.; Hori, M. & Yamasaki, Y. (2005). Post-prandial hyperglycemia is an important predictor of the incidence of diabetic microangiopathy in Japanese type 2 diabetic patients. *Biochemical & biophysical research communication*, 366:339-345.
- Sima, A. A. F. (2004). Diabetic neuropathy in type 1 & type 2 diabetes & the effect of C-peptide. *Journal of clinical sciences*, 220:133-136.
- Singh, S.K.; Sahay, R.K and Krishnam A. (2008). Oxidative stress in diabetic foot ulcer. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*. 2:109—113.
- Sorensen, L.; Molyneaux, L. & Yue, D.K. (2002). Insensate versus painful diabetic neuropathy: the effects of height, gender, ethnicity and glycaemic control. *Diabetes Research and Clinical Practice*, 57:45–51.
- Stamboulis, E.; Voumvourakis, K.; Andrikopoulou, A.; Koutsis, G.; Tentolouris, N.; Kodounis, A. and Tsivgoulis, G. (2009). Association between asymptomatic median mononeuropathy and diabetic polyneuropathy severity in patients with diabetes mellitus. *Journal of the Neurological Sciences*, 278:41–43.
- Takahashi, Y.; Tachikawa, T.; Ito, T.; Takayama, S.; Omori, Y. & Iwamoto, Y. (1998). Erythrocyte aldose reductase protein: a clue to elucidate risk factors for diabetic neuropathies

- independent of glycemic control. *Diabetes Research and Clinical Practice*, 42:101–107.
- Teitz, N. W. (2006). *Fundamentals of clinical chemistry*. 4th ed. Saunders, Philadelphia, p 948.
- Tesfaye, S. (2006). Neuropathy in diabetes. *Complications of diabetes*, 34(4):91-95.
- Tinggi, U. (2003). Essentiality and toxicity of selenium and its status in Australia: a review. *Toxicology Letters*, 137:103/110
- Tracy, J.A. and Dyck, J.P.B. (2008). The Spectrum of Diabetic Neuropathies. *Phys Med Rehabil Clin N Am.*, 19:1–26.
- Trinder, P. (1969). Determination of total serum cholesterol. *Analyst*. 76:596.
- Turgut, N.; Karasalihoglub, S.; kugurluoglub, Y.; Balcia, K.; Ekukluc, G. & Tuler F. (2004). Clinical utility of dorsal sural nerve conduction studies in healthy and diabetic children. *Clinical Neurophysiology*, 115:1452–1456.
- Uluc, K.; Isak, B.; Borucu, D.; Temucin, K.M.; Cetinkaya, Y.; Koytak, P.K.; Tanridag, T. and Us, O. (2008). Medial plantar and dorsal sural nerve conduction studies increase the sensitivity in the detection of neuropathy in diabetic patients. *Clinical Neurophysiology*, 119:880–885.
- Uncini, A.; De Angelis, M.V.; Di Muzioa, A.; Callegarini, C.; Ciucci, G.; Antonini, G.; Lugaresi, A. & Gambi, D. (1999). Chronic infalmmatory demyelinating polyneuropathy in

diabetics: motor conductions are important in the differential diagnosis with diabetic polyneuropathy. *A Clinical Neurophysiology*, 110:705+711.

Unger, J and Cole, B.E. (2007). Recognition and Management of Diabetic Neuropathy. *Prim Care Clin Office Pract.* 34:887–913.

Van Acker, K.; Bouhassira, D.; De Bacquer, D.; Weiss, S.; Matthys, K.; Raemene, H.; Mathieu, C. & Colin, I. M. (2009). Prevalence and impact on quality of life of peripheral neuropathy with or without neuropathic pain in type 1 and type 2 diabetic patients attending hospital outpatients clinics. *Diabetes & metabolism*, 30:30-38.

Van Dam, P.S.; Van Asbeck, B.S.; Bravenboer, B.; Van Oirshot, J.F.L.M. Gispen, W.H. and Marx, J.J.M. (1998). Nerve function and oxidative stress in diabetic and vitamin E-deficient rats. *Free Radical Biology & Medicine*, 24/1:18–26,

Vinik, A.I. (1999). Diabetic Neuropathy: Pathogenesis and Therapy. *The American journal of medicine*, 107:180-190.

Vinik, A.I.; Strotmeyer, E.S.; Nakave, A.A. & Patel, C.V. (2008). Diabetic Neuropathy in Older Adults. *Clin Geriatr Med*, 24:407–435.

Wamboldt, C. & Kapustin, J. (2006). Evidence based treatment of DPN. *The Journal for Nurse Practitioners*, 270-277.

Weintrob, N.; Amitaya, I.; Lilos, P.; Shalitina, S.; Lazar, L. & Josefsberg, Z. (2007). Bedside neuropathy disability score

compared to quantitative sensory testing for measurement of diabetic neuropathy in children, adolescents, and young adults with type 1 diabetes. *Journal of Diabetes and Its Complications* 21:13– 19.

Weiswasser, J.M.; Arora, S.; Shuman, C.; Kellicut, D. and Sidawy, A.N. (2003). Diabetic Neuropathy. *Seminars in Vascular Surgery*. 16:27-35.

Willems, D.; Dorchy, H. & Dufrasne, D. (1998). Serum antioxidant status and oxidized LDL in well-controlled young type 1 diabetic patients with and without subclinical complications. *Atherosclerosis*, 137 Suppl.:61–64.

Williams, B.; Gallacher, B.; Patel, H.; & Orme, C. (1997). Glucose-induced protein kinase C activation regulates vascular permeability factor mRNA expression and peptide production by human vascular smooth muscle cells in vitro. *Diabetes*, 46(9):1497–1503.

Wilson T.; Chawla, J. & Fisher, M. (2005). Sensitivity and specificity of electrodiagnostic criteria for CIDP using ROC curves: comparison to patients with diabetic and MGUS associated neuropathies. *Journal of the Neurological Sciences*, 231:19– 28

Wirotkiko, B.; Wong, T.Y. and Simo, R. (2008). Vascular endothelial growth factor and diabetic complications. *Progress in Retinal and Eye Research*. 30:1–14.

Yagihashi, S.; Yamagishi, S. & Wada, R. (2007). Pathology and pathogenetic mechanisms of diabetic neuropathy: Correlation

with clinical signs and symptoms. *Diabetes Research and Clinical Practice*, 77:184–S189.

Yasuda, H.; Sanada, M.; Kitada, K.; Terashima, T.; Kim, T.; Sakaue, Y.; Fujitani, M.; Kawai, H.; Maeda, K. and Kashiwagi, A. (2007). Rationale and usefulness of newly devised abbreviated diagnostic criteria and staging for diabetic polyneuropathy. *Diabetes Research and Clinical Practice*, 77:178–183.

Ziegler, D.; Sohr, C. G. & Nourooz-Zadeh, J. (2006). Oxidative stress and antioxidant defense in relation to the severity of diabetic polyneuropathy and cardiovascular autonomic neuropathy. *Diab. Care*, 27: 2178–2183.

Table 4.8a The values of lipid profile between patients & controls of type 1 diabetics according to the sex.

Variable		TC(mmol/L)		TG(mmol/L)		VLDL(mmol/L)		HDL(mmol/L)		LDL(mmol/L)	
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Type 1 DM	DPN patients	4.5±0.3	4.3±0.4	1.3±0.4	0.8±0.6	0.2±0.08	0.17±0.1	0.9±0.1	1.8±0.2	2.9±0.8	3±1
	Diabetics without neuropathy	4±0.6	4±0.3	1.1±1	1±0.4	0.1±0.03	0.15±0.1	1±0.6	1.3±0.4	2.5±1	2.8±2
Healthy control of type 1 DM		4.2±0.4	4±1	1±0.4	1.2±0.3	0.27±0.1	0.2±0.2	1.2±0.6	1.5±0.9	2±1.2	2.1±1

Table 4.8b- The values of lipid profile between patients & controls of type 2 diabetics according to the sex.

Variable		TC(mmol/L)		TG(mmol/L)		VLDL(mmol/L)		HDL(mmol/L)		LDL(mmol/L)	
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Type 2 DM	DPN patients	3.7±0.4	5.1±0.2	1.1±0.6	1.3±0.2	0.22±0.1	0.2±0.05	1.1±0.2	1.4±0.1	2.3±1	3.1±0.5
	Diabetics without neuropathy	4.3±0.7	4.3±1	0.8±0.6	1.1±0.6	0.16±0.1	0.2±0.07	0.6±0.2	1±0.1	2.8±1	2.6±0.6
Healthy control of type 2 DM		4.2±1	4.5±1.1	0.8±0.2	0.7±0.5	0.13±0.1	0.2±0.1	1.3±0.6	2±1.1	2.3±1	2.8±2

Vales are mean ± standard error, results are non significant.

DPN: diabetic peripheral neuropathy, DM: diabetes mellitus,

TC: total cholesterol, TG: triglyceride, VLDL: very low density lipoprotein, HDL: high density lipoprotein, LDL: low density lipoprotein.