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Molecular Insights and Pharmacotherapy of Diabetic Neuropathy

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Abstract

Background: Diabetes mellitus is a chronic disorder of energy metabolism caused by lack or decrease in the effectiveness of insulin, and is characterised by an abnormally elevated blood glucose concentrations and the development of macrovascular, microvascular and/or neuropathic complications. Diabetic neuropathy (DN) is one of the most debilitating outcomes of diabetes mellitus, and may cause pain, decreased mobility as well as amputation. Diabetes can damage the peripheral nervous system (PNS), through the induction of de-myelination in neurones, precipitated by chronic hyperglycaemia-induced oxidative stress, and causing a condition that involves the upper and lower limbs. ***Objective:*** The study discussed the risk factors for insulin resistance and pre-diabetes, type II diabetes mellitus and vascular complications, cellular and molecular basis of DN, physiopathological mechanisms, and the pharmacological treatment of DN. ***Method:*** The study examined journal articles and standard textbooks, as it relates to diabetes mellitus and its complications. Search for articles on DN was carried out in the literature. These were identified and reviewed for selection using chemical abstracts service, pubmed, google scholar, crossreference, web of science, pubmed central free article, and scopus. The key words used for search were: diabetic neuropathy; diabetic peripheral neuropathy; peripheral neuropathy; microvascular complications of diabetes mellitus; and neuromuscular complications of diabetes mellitus. ***Result & Discussion:*** Two hundred and fifty (250) articles and other works were identified, while ninety-seven (97) articles and non-journal materials were extracted and reviewed, taking into account the criteria for selection. Studies done in the last 4.5 decades were included, while works written on other languages, outside English were excluded. Findings indicate that DN is a complex disorder that affects the peripheral and/or cranial nerves, which is caused by unattended or poorly attended, long-term increase in blood glucose concentrations. It relatively manifests early, affecting a significant proportion of the micro-blood vessels in the middle-aged and elderly diabetic patients. DN causes numbness, loss of sensation, and sometimes pain in the feet, legs, arms or hands. Hyperglycaemia causes the activation and inhibition of several molecular pathways that are crucial for homeostasis in neuronal and neuroglial cells. ***Conclusion:*** DN is the commonest complication of diabetes mellitus. It has no known definitive therapy. Treatment is essentially symptomatic with huge economic and psychological burden, hence the rationale for a cost effective and targeted therapies. Achieving euglycaemia using anti-diabetic regimens (i.e., insulin and oral hypoglycaemic agents), foot care, changes in feeding habits and lifestyle modification are critical to holistically address the problem.

Keywords: Insulin Resistance, Pre-Diabetes, Hyperglycaemia, Oxidative Stress, Disorder.

INTRODUCTION

Diabetes mellitus is a chronic pathological syndrome of significant public health concern. It is a complex disorder of energy metabolism caused by lack or decrease in the effectiveness of insulin, and is always associated with abnormally elevated blood glucose concentrations, glycosuria, dyslipidaemia, end-organ damage or a negative nitrogen balance (Khanra *et al*., 2015; Maira, 2015; Bhattachargee *et al*., 2017; Khanra *et al*., 2017; Pearson and McCrimmon, 2018). The precise aetiology is not known, but it is generally believed to be as a result of the interplay between genetic susceptibility of the individual and environmental factors. Insulin lack affects the metabolism of macromolecules, including carbohydrates, proteins and fats, and produces a significant alteration of electrolyte and water balance in

the milieu interior (internal environment) of the organism, and thus disrupts the homeostatsis (Pearson and McCrimmon, 2018). Death caused by diabetes mellitus is due to acute metabolic decompensation (e.g., diabetic keto-acidosis - DKA and hyperglycaemic hyperosmolar state - HHS). DKA and HHS are two grave complications of diabetes mellitus, and are both referred to as hyperglycaemic crisis or decompensated forms of diabetes. Decompensated diabetes is a clinical entity consequent upon uncontrolled spike in blood glucose concentrations above 10 mmol/litre (or 180 mg/dl), which is characterised by hyperglycaemia. Long-standing metabolic derangement is almost always associated with functional and structural changes in the body cells, which can be permanent and irreversible, especially the vascular system. It is these molecular and pathological alterations that culminate in the complications of diabetes mellitus, which in turn, impact on the eyes, kidneys, and the nervous system. Diabetes can damage the peripheral nervous system, thereby making DN the commonest long-term complications of uncontrolled diabetes mellitus (Feldman *et al*., 2017). DN is a chronic and complex disorder that affects the peripheral nerves, causing condition that involves the upper and lower extremities (Callaghan *et al*., 2012; Jensen and Finnerup, 2014; Feldman *et al*., 2017) with an incidence rate of about 70 % in diabetic patients (Ghandhi and Selvarajah, 2015). The molecular mechanism(s) by which chronic hyperglycaemia leads to peripheral nerve injury is/are ill-understood. However, a number of biochemical pathways have been implicated in DN. They include, but not limited to the polyol (sorbitol) pathway (PP) - where the key cellular and molecular events occur, through the activation of aldose reductase, protein glycosylation, and the development of advanced glycation end-products (Thornalley, 2002; Yan, 2014; Schreiber *et al*.,2015); advanced glycation end pathway (AGE); protein kinase C pathway (PKC); hexosamine biosynthetic pathway (HBP); poly(ADP-ribose) polymerase pathway (PARP); mitogen-activated protein kinases pathway (MAPKs). These molecular pathways generate FRS coupled to oxidative stress, reduced neurotrophic support, and increased PKC activation, which culminate in peripheral nerve damage. As a consequence of the metabolic mismatch that underpins diabetes mellitus, mitochondrial failure and inflammatory processes often abound, which correlate with the phosphorylation of MAPKs (Younger *et al*., 1996; Satoh *et al*., 2003; Tomlinson and Gardiner, 2008; Yamagishi *et al*., 2008; Du *et al*., 2010).

Staging of Diabetes Mellitus

Diabetes mellitus has four (4) stages and occurs in the following chronological succession:

Stage 1: Insulin resistance
Stage 2: Pre-diabetes
Stage 3: Type II diabetes
Stage 4: Type II diabetes with vascular complications

Insulin resistance → pre-diabetes → type II diabetes → type II diabetes with vascular complications

Flow chart showing the developmental stages in diabetes mellitus

Insulin Resistance and Pre-Diabetes

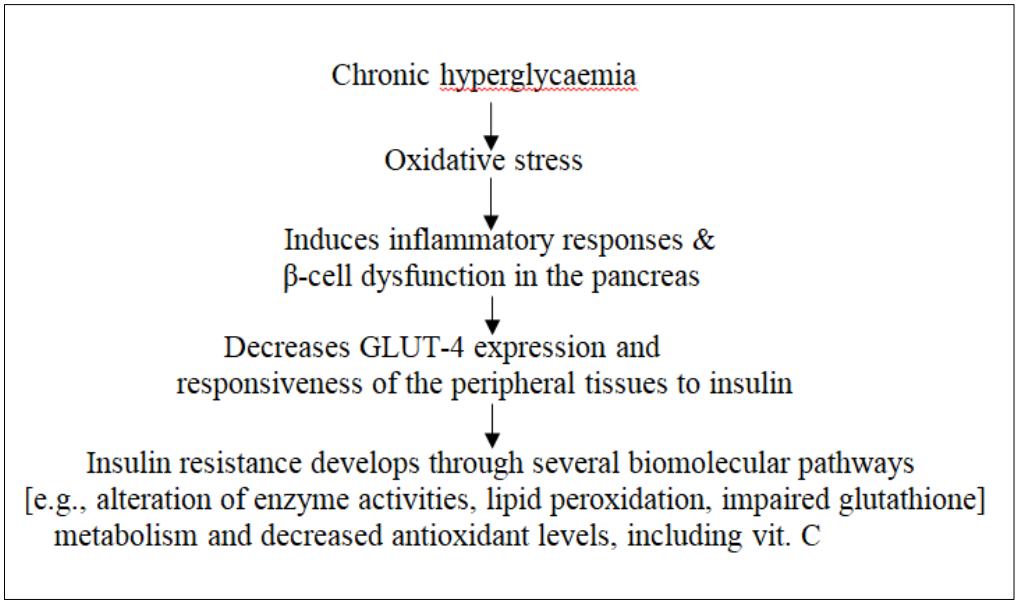

Fig. 1: Development Cascade of Insulin Resistance in the Peripheral Tissues

Insulin resistance is a complex metabolic problem whose precise molecular basis remains opaque. However, a reduction in the population of insulin receptors and post receptor signalling are believed to be the culprits. When insulin binds to its receptor, it induces the translocation of GLUTs (glucose transporters) to the cell membrane, thereby enhancing the uptake of glucose by the cells. Insulin resistance, therefore, refers to the

decrease or failure in the normal responsiveness of the peripheral or target tissues (e.g., skeletal muscle cells, fat cells and liver cells) to circulating insulin, which in turn, make these tissues unable to mop-up blood glucose (Maira, 2015). In other words, in times of insulin resistance, insulin cannot effectively direct blood glucose disposition leading to: failure to inhibit gluconeogenesis in the liver with increased fasting blood glucose concentrations; failure to stimulate glycogenesis in the skeletal muscle with raised post-meal blood glucose concentrations; and the failure to inhibit lipoprotein lipase enzyme in the adipose tissue with excess circulating free fatty acids. As a consequence, the β-cells of the pancreas become hyperactive and secrete more insulin into the blood causing hyperinsulinaemia in order to compensate for the insulin resistance, and which eventually push blood glucose into the cells (Maira, 2015). Insulin resistance and pre-diabetes have no classical clinical symptoms. Some individuals may present with darkened skin in the armpit or on the back and sides of the neck, a clinical condition called *acanthosis nigricans*. Skin tags (small skin growths) could be found in the same anatomical sites. Others include early changes in the eyes that may progress to retinopathy (a leading cause of preventable blindness caused by damage to the blood vessels (e.g., central retinal and short-posterior ciliary arteries) of the light-sensitive tissue at the back of the eye, the retina.

In pre-diabetes, the blood glucose is higher than normal (deranged), but not high enough to be diagnosed as diabetes mellitus. Pre-diabetes usually occurs in those with insulin resistance or whose β-cells are not producing sufficient insulin to maintain the blood glucose within normal or physiological limits. If insulin is not adequately produced and secreted into the blood, extra-glucose remains in the vascular system, instead of entering the cells, and diabetes may ensue.

Risk Factors for Insulin Resistance and Pre-Diabetes

Individuals with genetic or lifestyle risk factors are more prone to develop insulin resistance or pre-diabetes, and they include:

a) Obesity and overweight: Overweight is a body mass index (BMI) between 25–29. Truncal obesity, which is the excess accumulation of fats in the chest, abdomen and the pelvis in an obese with a BMI of ≥ 30 kg/m^2 [or a waist circumference of > 40 inches (> 101.6 cm) for men and > 35 inches (> 88.9 cm) for women]. Truncal obesity and visceral fats are the main culprits in insulin resistance.
b) Age 45 and above
c) Familial
d) Race: African Americans, American Indians, Hispanics/ Latinos, etc. These racial groups have higher risk for developing insulin resistance or pre-diabetes.
e) Physical inactivity (sedentary lifestyle): This is linked to insulin resistance.
f) Hypertension
g) Hypercholesterolaemia
h) Heart disease
i) Stroke
j) Polycystic ovarian syndrome: A gynaecological disorder where the ovaries produce excess amounts of androgens and causing imbalance in the female reproductive hormones. This manifests with irregular menstrual cycles (involving missed periods, unpredictable ovulation or anovulation, heavy menstruation - menorrhagia), abnormal hair growth (hirsutism), acne, obesity, hyperpigmentation (darkening of the skin), small follicle cysts, skin tags, thinning hair/ baldness, and infertility.
k) Metabolic syndrome (syndrome X, insulin resistance syndrome or dysmetabolic syndrome): This is a group of clinical conditions, which includes high blood pressure, truncal obesity, high blood triglycerides, low levels of HDL cholesterol, and insulin resistance/ high blood glucose concentrations. Metabolic syndrome is a risk factor for cardiovascular disease.
l) Drugs (e.g., anti-retrovirals, corticosteroids and anti-psychotics)
m) Cushing's syndrome and acromegally
n) Sleep apnoea

Diagnosis of Insulin Resistance and Pre-Diabetes

Laboratory investigations for insulin resistance are complicated and mostly employed in experimental diabetology research. A haematological screening can be performed in the hospital to determine the presence or otherwise of pre-diabetes. Tests that can be done include:

1. **Fasting plasma glucose (FPG):** The patient fasts for at least 12 h, and fasting usually begins post supper. FPG of 100 – 125 mg/dl (5.6 – 6.9 mmol/l) depicts pre-diabetes.

2. **Glycosylated/ Glycated Haemoglobin (HbA1c) Test:**

This test measures the amount of glucose in the blood in the preceding 2 – 3 months. The test is often known as $A1_C$ test or sometimes $HbA1_C$ test. It is a haematological investigation employed to detect the presence of pre-diabetes (i.e., high blood glucose concentrations that can progress to diabetes, heart disease, and stroke); diagnosis of diabetes mellitus; and also to determine the degree, extent or quality of management of the disease (Cleveland Clinic, 2021). $A1_C$ test relies on haemoglobin, the protein component of the red blood cell that carries oxygen round the body. The presence of glucose in the blood causes glucose to stick to haemoglobin, a condition referred to as *glycosylation or glycation*. The more the glucose in the blood, the greater the glycation process. $HbA1_C$ test is reported as a percentage. The test is done 2 – 4 times per year. The higher the percentage of the glycated haemoglobin, the higher the blood glucose concentrations have been in the

last few months. Less than 5.7 % means no diabetes; 5 – 7 % signals pre-diabetes; ≥ 6.5 is diagnostic of diabetes mellitus. ≤ 7 is the therapeutic objective or goal for the management of diabetes mellitus (Cleveland Clinic, 2021).

3. Oral Glucose Tolerance Test (OGTT):

The test is expensive, and shows the blood glucose concentration at the time of investigation. It is a more sensitive test relative to $HbA1_C$ test, which sometimes may not detect pre-diabetes. It measures how the body handles postprandial glucose, and often employed in the diagnosis of gestational diabetes. Individuals with pre-diabetes have 50 % probability of developing diabetes mellitus within 5 – 10 years. The presence of one or more risk factors is a pre-requisite for a regular monitoring for diabetes. Even when results are within acceptable normal (physiological) limits, and there is the presence of other risk factors, a repeat test should be conducted tri-annually (i.e., every three years).

Table 1: WHO Diagnostic Criteria for Diabetes Mellitus

Test Normal	Impaired glucose tolerance	Diabetes mellitus
FPG < 5.6 mmol/l <100.0 mg/dl	5.6 – 6.9 mmol/l 100 – 125 mg/dl	≥ 7.0 mmol/l ≥ 126.0 mg/dl
RPG < 7.8 mmol/l < 140.0 mg/dl	7.8 – 11.0 mmol/l 140.0 – 199.0 mg/dl	≥ 11.1 mmol/l ≥ 200.0 mg/dl
2HPP < 7.8 mmol/l <140.0 mg/dl	7.8 – 11.0 mmol/l 140.0 – 199.0 mg/dl	≥ 11.1 mmol/l ≥ 200.0 mg/dl
HbA1c < 5.7 % <39.0mmol/mol	5.7 – 6.4 % 39.0–46.0 mmol/mol	≥ 6.5 % ≥ 47.0 mmol/mol

Source: World Health Organisation (2019)

FPG - fasting plasma glucose; RPG – random plasma glucose; 2HPP – two hours postprandial or postglucose meal; HbA1c - glycosylated or glycated haemoglobin

Table 2: Hyperglycaemic Crises Presentation in Diabetes Mellitus

Diagnostic Parameter	Diabetic Ketoacidosis (DKA)	Hyperglycaemic Hyperosmolar State (HHS)
Plasma glucose	> **250.0 mg/ml**	> **600.0 mg/dl**
Arterial pH	≥ 7.3	> 7.3
Serum HCO_3^-	>18.0 Meq/l	>18.0 Meq/l
Ketonuria	Positive	Small
Ketonaemia	Positive	Small
Effective serum osmolality	Variable	> 320.0 MOsm/l
Anion gap	≥ 10.0 Meq/l	Variable
Mental status	Alert to coma	Stupor to coma

Source: Pitman and winter (2021)

Prevention and Reversal of Insulin Resistance and Pre-Diabetes

1. Increase in physical activity and avoiding sedentary lifestyle: Regular exercises in moderation with few steps, including climbing stairs and healthy (adequate) diets rich in antioxidants (e.g., fruits and vegetables).
2. Weight reduction: Losing weight can reverse insulin resistance, and prevent or delay the development of type II diabetes. A reduction of 5 – 7 % of the starting weight can reduce the probability of developing type II diabetes mellitus (Luchsinger et al., 2017).

Type II Diabetes with Vascular Complications

Type II Dm is the most common type of diabetes. It is one of the most common metabolic disorders caused by a combination of two primary factors: defective insulin secretion by β-cells of the pancreas, and the inability of the insulin-sensitive tissues to respond appropriately to insulin (Galicia-Garcia *et al*., 2020). Vascular complications of type II Dm are the most serious manifestations of the disease, and remains a huge medical problem worldwide. Atherosclerosis is the main reason for impaired life expectancy in patients with type II Dm (as diabetes mellitus facilitates atheromatous plaques formation, a condition known as atherogenesis), whereas diabetic nephropathy and retinopathy are the largest contributors to end-stage renal disease and blindness respectively. Intensive lowering of blood glucose to achieve euglycaemia or normoglycaemia decreases the risk of microvascular disease, such as nephropathy and retinopathy; antihypertensive agents lower the risk of cardiac and vascular pathologies, nephropathy and retinopathy; pan-retinal photocoagulation and agents that target vascular endothelial growth factor (VEGF) slow the progression of diabetic retinopathy; while the use of HMG CoA reductase inhibitors (statins) decrease the risk of heart and blood vessel diseases (Rask-Madsen and King,

2014). Adults with type II Dm have an annual mortality of about 5.4 %, and their life expectancy is decreased on average by 5 – 10 years (Donnelly *et al*., 2000). Many of the deaths are due to cardiovascular disease; deaths from non-cardiovascular disease are also increased. Diagnosis of type II Dm increases the risk of developing a number of clinical complications which are irreversible and due to microvascular and macrovascular disease. Chronicity or duration of diabetes is a critical factor in the pathogenesis of complications, but other risk factors (e.g., hypertension, cigarette smoking, obesity and hypercholesterolaemia) interact with diabetes mellitus to affect the clinical course of microangiopathy and macroangiopathy. Progression of vascular complications of type II Dm depends on the severity and duration of hyperglycaemia (Donnelly *et al*., 2000).

Table 3: Therapeutic Target Values in Diabetes Mellitus

Type of Investigation	Controlled Diabetes	Uncontrolled Diabetes
Fasting blood glucose	**< 6.0 mmol/l** **< 108.0 mg/dl**	**> 8.0 mmol/l** **> 144.0 mg/dl**
2 hour post meal (glucose)	< 8.0 mmol/l < 144.0 mg/dl	> 10.0 mmol/l > 180.0 mg/dl
HbA1c	< 7.0 % < 51.7 mmol/mol	> 8.0 % > 59.1 mmol/mol

Source: Nwonu and Nwonu

Table 4: Vascular Complications of Diabetes Mellitus

Microvascular	Macrovascular
Retinopathy	**Ischaemic heart disease (e.g., angina pectoris, myocardial infarction)**
Nephropathy	Cerebrovascular disease (e.g., ischaemic stroke, haemorrhagic stroke)
Neuropathy	Peripheral vascular diseases (e.g., peripheral artery disease, peripheral venous disease)

Source: Donnelly *et al*., (2000)

Table 5: Risk of Morbidity Associated with all Types of Diabetes Mellitus

Complication	Relative Risk** (compared with non-diabetic patients)
Blindness	**20**
End-stage renal disease	**25**
Amputation	**40**
Myocardial infarction	**2 - 5**
Stroke	**2 – 3**

Source: Donnelly *et al*., (2000)

Hypertension affects at least 50 % of patients with type II Dm. Hypertension and smoking also have adverse effects on microvascular outcomes. Type II Dm is associated with a more atherogenic lipid profile, especially low concentrations of HDL cholesterol and high concentrations of small, dense, LDL particles. A mean blood pressure of 144/82 mmHg will achieve significant reductions in the risk of stroke (44 %), heart failure (56%), diabetes- associated deaths (32 %), including microvascular complications (e.g., 34 % reductions in the progression of retinopathy). About 30.3 % of patients will require a combination of therapy for the management of their hypertension (i.e., about two or more antihypertensive medications in order to maintain a target blood pressure of less than 150/ 85 mmHg (Donnelly *et al.*, 2000).

Endothelial Dysfunction in Diabetes Mellitus: Molecular Perspectives

Endothelial dysfunction is a mismatch in the production of vascular factors, which when disrupted predisposes the blood vessels to pro-thrombotic and pro-atherogenic effects, thus leading to vasoconstriction, leucocyte adherence, platelet activation, mitogenesis, pro-oxidation, impaired coagulation and nitric oxide production, vascular inflammation, atherosclerosis, and thrombosis. Endothelial dysfunction is a key contributing factor in the aetiopathogenesis of vascular disease in diabetes mellitus (Dhananjayan *et al*., 2016). Progression of vascular complications of type II Dm depends on the severity and duration of hyperglycaemia (Donnelly *et al*., 2000). Endothelial dysfunction is a potential therapeutic drug target for individuals with type II Dm, and also plays a critical role in atherosclerosis progression leading to cardiovascular complications. There is a significant association between type II Dm, oxidative stress and endothelial dysfunction (Maruhashi and Higashi, 2021). A major step in the development of endothelial dysfunction caused by abnormally elevated blood glucose is oxidative stress, which stem from the cytoplasm and in the mitochondria of the cell. Oxidative stress ensues when the rate of production of reactive oxygen and nitrogen species (e.g., $O_2^{\bullet -}$, H_2O_2, NO, NO_2) in a cell predominates over their rate of utilisation and conversion to more stable products causing cellular and tissue damages. The disequilibrium between pro-oxidant and antioxidant ratio, which tilts towards pro-oxidant activity produces a change in the normal redox signalling

in the cell, and thereby results in the impairment of myriads of molecular pathways during cell metabolism (Graves *et al*., 2007). Oxidative stress, therefore, causes a mismatch between the FRS generated during metabolic processes and antioxidants defence system in the body. These reactive species impair cell regeneration and repair, and eventually speed up ageing process and development of diseases, including diabetes over a certain period of time. Endothelial cells have the potential to generate FRS from several enzymatic sources (e.g., phosphoinositide-3-kinase, mitogen-activated protein kinase, extracellular signal-regulated kinase, etc.). Chronic hyperglycaemia, glucose fluctuations and insulin resistance are involved in endothelial dysfunction. Mechanisms that increase oxidative stress and cellulopathy in the endothelium during diabetes include, elevated hyperglycaemia-induced FRS, both from cytosolic and mitochondrial sources; change on the glycolytic flow to alternate metabolic pathways (e.g., polyol pathway, hexosamine pathway, etc.) caused by hyperglycaemia; blockade of pentose-phosphate pathway - PPP (also known to as the hexose monophosphate pathway or shunt) triggered by abnormally high blood glucose levels; formation of glycation products and advanced glycation end-products; and the activation of protein kinase C (Maruhashi and Higashi, 2021). The PPP is a crucial metabolic route, and takes off from glucose-6-phosphate in the pathway of glycolysis, where it generates NADPH and ribose-5-phosphate, and in turn shunts carbon back to the glycolytic or the gluconeogenic pathway. Both NADPH and ribose-5-phosphate are vitally important for the survival and proliferation of cells. NADPH is the reducing power necessary for the biosynthesis of fatty acids, sterols, nucleotides and non-essential amino acids, whereas ribose-5-phosphate is the building block required for the biosynthesis of nucleic acids. The PPP regulates intracellular redox (oxidation/ reduction) homeostasis and biosynthesis (Wamelink *et al*., 2008; Patra and Hay, 2014).

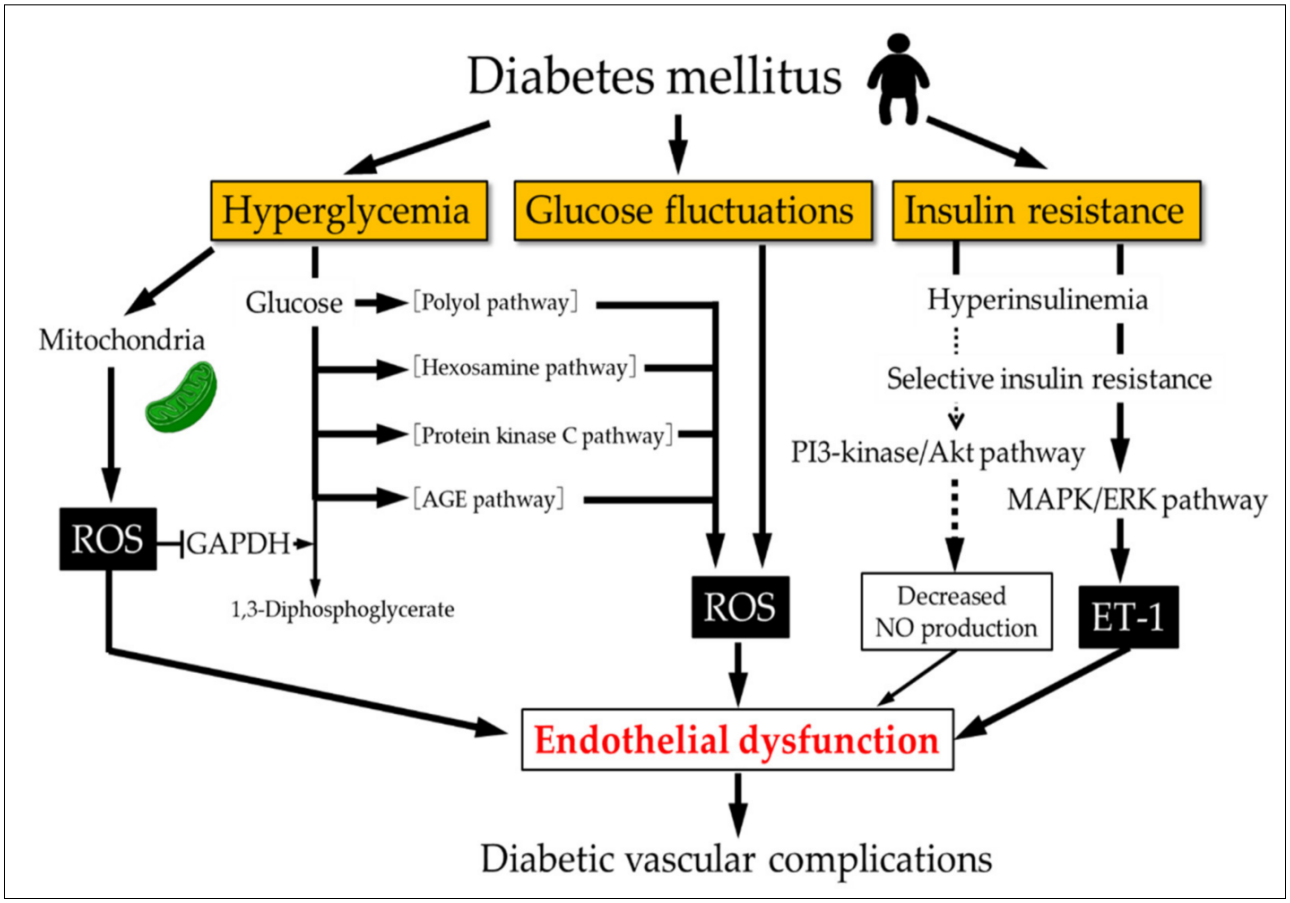

Fig. 2: Mechanism of Endothelial Dysfunction in Chronic Diabetes Mellitus
Source: Maruhashi and Higashi (2021)

ROS (reactive oxygen species), GAPDH (glyceraldehyde-3-phosphate dehydrogenase), ET-1 (electron transport-1), PI_3-Kinase/ Akt pathway (phosphatidylinositol-4,5-bisphosphate-3-kinase/ protein kinase B pathway), MAPK/ ERK pathway (MAPK, mitogen-activated protein kinase), ERK (extracellular signal-regulated kinases).

DIABETIC NEUROPATHY

DN is a degenerative nerve disorder or nerve damage that affects the PNS and has become an

important public health crisis globally. Clinically, DN is defined as the presence of symptoms and/or signs of peripheral nerve dysfunction in persons with diabetes mellitus after other causes have been excluded (Boulton *et al*., 2005). It is a demonstrable disorder, either clinically evident or sub-clinical, that occurs in the setting of prolonged diabetes mellitus without other causes of peripheral neuropathy, and so, DN is a diagnosis of exclusion. The neuropathic disorder includes manifestations in the somatic and/or autonomic part of the peripheral nervous system (American Diabetes Association, 1988; American Academy of Neurology Consensus Statement, 1988). DN is one of the most debilitating outcomes of diabetes mellitus, and may cause pain, decreased mobility as well as amputation (Dewanjee *et al*., 2018). DN has been described as the most deceptive and least understood complications of diabetes, and is more difficult to treat relative to other diabetic pathological sequelae (Veves and King, 2001). The global prevalence of DN is approximately 30 % in hospitalised patients while community-based diabetic patients accounts for about 20 – 30 % (Papanas and Ziegler, 2015). DN significantly impacts on the peripheral nerves (e.g., somatic sensory nerves, motor nerves, and the autonomic nerves) in patients with chronic diabetes mellitus. DN first targets the sensory and autonomic axons, and subsequently, the motor axons, which progressively leads to sensory loss (Kaur *et al*., 2023). It can affect any part of the peripheral nervous system or many of the communication networks that transmit vital information from the central nervous system (CNS) to the body, and vice versa. Nerves that emanate from the spinal cord into the arms, hands, legs and feet are usually affected, but those extending specifically from the spinal cord to the feet are the most affected. Cranial nerves 3 and 6 are affected in long-term complications of diabetes mellitus, leading to diplopia or double vision (Pearson and McCrimmon, 2018). Other cranial nerves affected include 4, 5, 7, 9, and 10. Cranial nerve mononeuropathies of the III and VII nerves are the most common cranial nerve disorder in individuals with diabetes mellitus. DN is the most common and intractable long-term complications of diabetes mellitus (Boulton *et al*., 2005; Strotmeyer *et al*., 2009). It is the commonest type of nerve damage seen in people with diabetes, and specifically affects the micro-blood vessels. It arises due to metabolic impairment or a reduction in blood circulation which impacts on the peripheral and/or the cranial nerves. It is very difficult to determine which of the above is responsible for DN in a diabetic patient (American Diabetes Association, 2002). Elevated blood glucose concentrations (hyperglycaemia) damage the small blood vessels (micro-angiopathy) that supply the nerves in the body. As a consequence, essential nutrients conveyed *via* the vascular system fail to reach the nerves, thereby leading to nerve fibre damage or its disappearance. DN is relatively early in onset and affects 7 % of patients within 1-year of diagnosis up to 50 % for individuals with diabetes for more than 25-years (Pirart, 1978; Kaur *et al*., 2023). The prevalence of manifestation depends on the chronicity/duration of diabetes mellitus and the degree of metabolic control. The reduced longevity and the resultant high mortality rates in the diabetics are consequences of cardiovascular autonomic neuropathy. Loss of sensation (or feeling) in the lower limbs is a high risk for limb amputation, which occurs in 1 - 2 % of diabetic patients (Vinik and Ziegler, 2007). The major predictors of DN are the duration of diabetes and the blood concentrations of $HbA1_C$ (Tesfaye *et al*., 2005). The severity of DN correlates well with the severity of hyperglycaemia, an indication that DN is induced by excess blood glucose concentrations (Diabetes Control and Complications Trial Research Group, 1993).

Classification of Diabetic Neuropathy

The classification is based on the clinical presentation in the patients (Pop-Busui *et al*., 2017).

1. **Generalised Neuropathies**
   a) Distal symmetrical polyneuropathy
   b) Autonomic neuropathy (cardiovascular, gastrointestinal, urino-genital, sudomotor)
   c) Acute sensory neuropathy (treatment-induced neuropathy, diabetic neuropathic cachexia)

2. **Focal Neuropathies**
   a) Isolated cranial neuropathies (cranial nerves 3, 4, 6, 7, etc.)
   b) Isolated peripheral nerve neuropathies (e.g., ulna, median, femoral, peroneal nerves)
   c) Entrapment neuropathies (carpal tunnel syndrome, ulna neuropathy)
   d) Other mononeuropathies (phrenic mononeuropathy)

3. **Multi-Focal Neuropathies**
   a) Diabetic radiculoplexus neuropathy
   b) Lumbosacral radiculoplexus neuropathy
   c) Thoracic radiculoneuropathy
   d) Cervical radiculoplexus neuropathy

1. Distal Symmetrical Polyneuropathy

Distal symmetrical polyneuropathy (DSP) is otherwise known as peripheral neuropathy. Early signs are largely sensory, and they include loss of vibration sense, loss of pain sensation, and loss of temperature sensation in the feet. In later stages, the patient feels like he/she is '*walking on cotton wool'*, loss of balance or position, especially while trying to wash the face or walk in the dark due to impaired propioception or kinesthesia (i.e., impaired ability to sense movement, action or location). DSP does not usually affect the hands in the initial stages. Complications of DSP include unrecognised pedal trauma due to loss of pain sensation that may lead to infection, hence the need for regular examination of the feet by the patient. Another is atrophy (wasting) of the small muscles of the feet supplied by motor nerves. DSP fizzles out within few months to one-year with a good glycaemic control.

2. Autonomic Neuropathy

This is a common form of peripheral neuropathy seen in diabetics due to damage to the autonomic nerves, which control parts of the body whose movements are not under voluntary control (American Diabetes Association, 2002). Autonomic neuropathy is associated with increased mortality rate, as severe autonomic neuropathy requires treatment (Marsden and Fowler, 1998). The cardinal symptoms are postural hypotension (≥ 30 mmHg drop in systolic blood pressure on standing from a supine position causing light headedness or dizziness), abnormal sweating (too much or too little sweating or very dry skin), impotence (difficulty in achieving erection even when there is sexual desire), gastroparesis (delay in gastric emptying when there is no evidences of mechanical obstruction) and its sequelae – nausea, vomiting, constipation and diarrhoea, and decreased awareness of hypoglycaemia (hypoglycaemia unawareness). Other symptoms include, urinary tract infection arising from damage to the neuronal supply to the bladder and causing detrussor muscle weakness with incomplete emptying of the urinary bladder, vaginal dryness and decreased coital responses (American Diabetes Association, 2002).

3. Carpal Tunnel Syndrome

Carpal tunnel syndrome (CTS) or median nerve mononeuropathy is a rare type of focal neuropathy caused by damage to a single nerve or group of nerves. It is the most common of the entrapment neuropathies (i.e., neuropathies caused by pressure or repetitive injury to a peripheral nerve). The condition is relatively common in women. CTS arises from pressure on the median nerve in the fibro-osseous carpal tunnel at the wrist (Marsden and Fowler, 1998; Kirshner, 2007). Median nerve is supplied from the cervical roots C6, 7, 8 and T1 (C6 – T1). It is associated with repetitive use of the hands or flexing of the wrist for a very long time, and seen especially among typists, telephone operators, cobblers, seamstresses, factory and manual workers (Kirshner, 2007; Nowak et al., 2023). Risk factors that increase the development of CTS include, age (risk rises with age), women with narrow carpal tunnel (and are thrice affected than men), rheumatoid arthritis, diabetes mellitus, myxoedema (hypothyroidism), acromegally, amyloid deposition (amyloidosis) in the tissues around the median nerve, myeloma, pregnancy (third trimester), osteoarthritis, obesity, dysmetabolic syndrome, gout, previous trauma to the wrist involving the median nerve, as in Colles' fracture (Marsden and Fowler, 1998; Holt, 2021; Nowak *et al*., 2023). The earliest observable clinical manifestations of CTS are pain and tingling, or paraesthesia. These symptoms occur in the distribution of median nerve of the hand, including the palm, thumb, index and middle fingers, and median half of the ring finger. These sensory upset or symptoms metastasize up the forearm, but not usually above the elbow. The symptoms of CTS are aggravated by prolonged wrist flexion or repetitive use due to manual labour. Symptoms may awaken patient from sleep or appear on waking, or with the lifting of objects and carrying them in a specific position or while driving. These symptoms are relieved by hanging the hand down, moving it about or changing position. If CTS is left unattended to, motor weakness in the distribution of median nerve may ensue, with poor opposition of the thumb, weakness, and wasting (atrophy) of the thenar eminence (thenar pad muscles) of the hand. These weak muscles are principally the opponens pollicis and abductor pollicis brevis (Kirshner, 2007). Sensory changes may appear at the tip of the thumb, index, middle, and ring fingers. In some patients, the physician may not elicit any abnormal signs until the lesion becomes very severe (Marsden and Fowler, 1998; Kirshner, 2007). CTS is not usually a permanent condition. It sometimes disappears within 2-weeks to 18-months, even without treatment, provided the blood glucose is adequately under control (American Diabetes Association, 2002).

4. Mononeuropathy Multiplex

Mononeuropathy multiplex (mononeuritis multiplex) is an unusual (rare) form of peripheral neuropathy. Patients with diabetes mellitus have a predisposition to multiple mononeuropathies which can involve a combination of individual nerves (Kirshner, 2007). It is a painful, asymmetrical, asynchronous sensory and motor peripheral neuropathy involving isolated damage to at least two distinct nerve trunks, with multiple nerves, which may include cranial nerves in random locations in the body (Zhang et al., 2015). For instance, there can be a combination of ulnar neuropathy, median neuropathy and thoracic radiculopathy. Radiculopathy means the mechanical compression of the nerve roots as they exit the spinal or vertebral column. Isolated nerve palsies supplying the extra-ocular muscles of the eye, especially cranial nerves 3 and 6 are particularly affected in diabetes mellitus (Holt, 2021). The onset is usually sudden and can be painful. In cranial nerve 3 lesion, it is painless with intact pupillary reflex, because of the sparing of the pupillo-motor fibres. Spontaneous and complete recovery occurs within 3 – 6 months in most episodes of multi-focal neuropathy (Holt, 2021).

Risk Factors for Diabetic Neuropathy

1. Duration of diabetes: The longer or chronicity of the disease, the more likelihood of the patient developing peripheral neuropathy, especially in poorly controlled cases.
2. Age of patient: Peripheral neuropathy is more common in the middle aged and the elderly diabetic patient.
3. Glycosylated/ glycated haemoglobin $A1_C$ ($HbA1_C$): A test result of $A1_C$ that is between 5.7 – 6.4 signals pre-diabetes, while $A1_C$ test of 6.5 or above is diagnostic of diabetes mellitus, and thus signals poor diabetic control, including nerve damage.
4. Diabetic retinopathy: This is a complication of diabetes that affects the eye due to damage to

the vascular supply (central artery and short posterior ciliary artery) of the light-sensitive tissue at the back of the eye (i.e., the retina).

5. Smoking: Cigarette smoking narrows and hardens the tunica media of elastic arteries, and decreases the vascular supply to the lower extremities, especially the legs and feet. This makes it difficult for wound healing to take place, and eventually damages the peripheral nerves.
6. Overweight: A BMI of greater or equal to 25 (over-weight) increases the risk of diabetic neuropathy.
7. Renal pathology: Diabetes mellitus has the potential to instigate renal disease and damage the kidneys, and thus secretes toxins into the blood, which in turn damages the nerves.

Staging of Diabetic Neuropathy

There are five (5) clinical stages of DN, and they include:
Stage 1: Sporadic pain and numbness
Stage 2: Constant pain
Stage 3: Intense pain
Stage 4: Complete and constant numbness
Stage 5: Absolute loss of sensation

Clinical Manifestations and Complications of Diabetic Neuropathy

The distribution of the symptoms depends on the site of the peripheral nerve disease. Mononeuropathy (i.e., single nerve lesions) has localised symptoms while polyneuropathy (i.e., multiple nerve lesions) affects the limbs diffusely, usually with a distal predominance. In polyneuropathy, the longest nerves (i.e., nerves that extend from the spine to the feet) are mostly affected or typically manifest the most symptoms. Diabetic polyneuropathy affects multiple peripheral sensory and motor nerves that branch out from the spinal cord into the arms, hands, legs and feet. Clinical symptoms include: numbness (decreased feeling of pain) – numbness of the feet, with some involvement of the hand is typical, often referred to as a *stocking-glove* distribution, muscle weakness, fatigue, muscle twitches, cramps, arching, tingling, sharp and burning sensation sometimes worse at night in the toes, dry and cracked skin, loss of coordination (sensory ataxia).

The earliest signs of DN are decreased distal vibration sense and decreased touch (sensory loss), pins and needles sensation, hyperpathia (increased or exaggerated reaction to stimuli – touch, vibration, pinpricks, heat, cold or pressure), loss of ankle reflex (areflexia or hyporeflexia), and sensory ataxia.

Some of the complications of DN include:

1. **Hypoglycaemia Unawareness**

Blood glucose concentration <70 mg/dl (3.9mmol/l) triggers tremors, sweating and tachycardia. However, individuals with autonomic neuropathy may not present with these features of hypoglycaemia.

2. **Amputation**

Damage to the nerves causes loss of sensation in the feet, such that injuries may occur without being noticed until it progresses to sores and ulcers necessitating amputation of the limb.

3. **Urinary Tract Infection**

Damage to the innervation of the urinary bladder leads to poor or incomplete emptying of the bladder. This causes aggregation of bacteria in the bladder and the kidneys leading to urinary tract infection.

4. **Urinary Incontinence**

Nerve damage leads to loss of the ability to sense the urge to urinate or control the detrussor muscle of the urinary bladder. As a consequence, there is incontinence of urine.

5. **Hypotension**

Damage to the nerves that control blood flow can affect the ability to regulate blood pressure in the body. This causes a sudden reduction in the blood pressure in an attempt to stand after sitting or lying in a supine position, which may result in lightheadedness (dizziness) and fainting spells or attack (syncope).

6. **Alimentary Disorders**

Constipation, diarrhea or both may ensue when the nerve supply to the gut is damaged. Diabetes-associated nerve damage can cause gastroparesis (i.e., arrest of downward movement of gastric contents by peristaltic waves without any evidences of mechanical obstruction). The resultant effect is bloating and indigestion.

7. **Diaphoresis**

DN is associated with abnormal sweating. Sweating may either increase or decrease due to peripheral nerve damage. This leads to malfunctioning of the sweat glands, thereby making it difficult to adequately control the body temperature.

Prevention of Diabetic Neuropathy

1. Blood glucose measurement: This is determined using the $A1_C$ test by estimating the quality of diabetic control in the previous 2 – 3 months.
2. Foot care: Foot problems (e.g., foot ulcers, sores and limb amputation) are the commonest complications of diabetic neuropathy. It can be prevented by regular feet examination looking for blisters, cuts, bruises, cracked and peeling skin, erythema, and swelling.

As part of care of the feet, the following measures must be taken care of:

i) The feet have to be clean and dry at all times.
ii) Daily examination of the feet.
iii) Moisturisation of the feet.

iv) Careful trimming of the toe nails to avoid striking the toe or toe nails against a sharp or solid object and injuring oneself.

CELLULAR AND MOLECULAR BASIS OF DIABETIC NEUROPATHY

Oxidative stress is the foundation of insulin resistance, diabetes, cardiovascular diseases, and many other age-related and degenerative diseases (Rains and Jain, 2011; Yashin *et al*., 2017; Yaribeygi *et al*., 2020). Hyperglycaemia causes a reduction in glycolysis and places a heavy metabolic burden on the tricarboxylic acid (TCA) cycle resulting in oxidative stress. Uncontrolled diabetes mellitus with consistent hyperglycaemia is the trigger of diabetic peripheral neuropathy, a common neuromuscular complication of diabetes mellitus, which manifests with constipation, muscle weakness, tingling and burning sensations, numbness, hyperalgesia (abnormally increased sensitivity to pain due to damage to the nociceptors or the peripheral nerves), etc. Constant hyperglycaemia causes the activation, and the inhibition of various biochemical pathways that play critical roles in the homeostasis of neuronal cells, glial cells and others. Impaired glucose metabolism (i.e., chronic hyperglycaemia with accumulation of glucose and glycolytic intermediates) is an important mechanism that induces oxidative stress due to shunting of excess glucose to other metabolic and non-metabolic pathways, resulting in the activation of a host of cellulo-molecular pathways, including sorbitol, hexosamine, advanced glycation end pathway amongst others. These shunting processes precipitate mitochondrial injury with high rates of protons returning to the mitochondria without generation of ATP, and an upsurge in the production of FRS in the neurone (Fernyhough and McGavock, 2014). Superoxide radical ($O_2^{\bullet -}$) inhibits glyceraldehyde-3-phosphate dehydrogenase activity, which is believed to be the cause of accumulation of all glycolytic intermediates. Inhibition of the glycolytic pathway triggers the hexose monophosphate shunt (pentose phosphate pathway), thereby generating NADPH, which is employed in the polyol pathway. Hyperglycaemia thus increases the production of FRS. Within physiological limits, glucose is converted to glucose-6-phosphate in the cells, then glycolysis, and oxidation to yield NADH and acetyl CoA. Electrons that are carried by NADH are transferred to oxygen following mitochondrial electron transport chain, including the pumping of protons out of the mitochondrial matrix. This creates a proton gradient used by ATP synthase to generate ATP, and converting oxygen (O_2) to water (H_2O). In DN, glucose metabolism is disrupted, leading to the accumulation of glucose and glycolytic intermediates, causing mitochondrial injury, and resulting in the conversion of O_2 to $O_2^{\bullet -}$, instead of H_2O. As a consequence of the overwhelming production of superoxide radical amongst others, ATP production is markedly diminished. Minute amounts of electron leaking out of the mitochondrial matrix is used for the production of FRS under physiological conditions (Turrens, 2003). Defective mitochondrial glucose oxidation has been fingered as the main culprit in the production of FRS (Brownlee, 2001; Brownlee, 2005), and neurones have inherent, sufficient capacity to neutralise and eliminate FRS through endogenous/autogenous cellular antioxidant mechanism (Nishikawa *et al*., 2000). Hyperglycaemia, dyslipidaemia, insulin resistance and its sequelae, induce alterations in the mitochondrial function, inflammation, oxidative stress, specific gene transcription and expression, and the eventual activation of myriads of signalling pathways, which constitute the major source of neuronal and neuroglial cell damage, and these cellulo-molecular pathways constitute potential therapeutic targets in diabetic neuropathy (Sanaye *et al*., 2023; Zhu et al., 2024). Disruption of these biochemical pathways leads to apoptosis (axonal myelin sheath injury) and dysfunction in the mitochondria, and thereby results in neuropathy (Sanaye *et al*., 2023). Biomolecular pathways implicated in the development and progression of DN include, but not limited to protein kinase C pathway, polyol pathway, advanced glycation end pathway, hexosamine biosynthetic pathway, poly (ADP-ribose) polymerase pathway, and mitogen-activated protein kinase pathway.

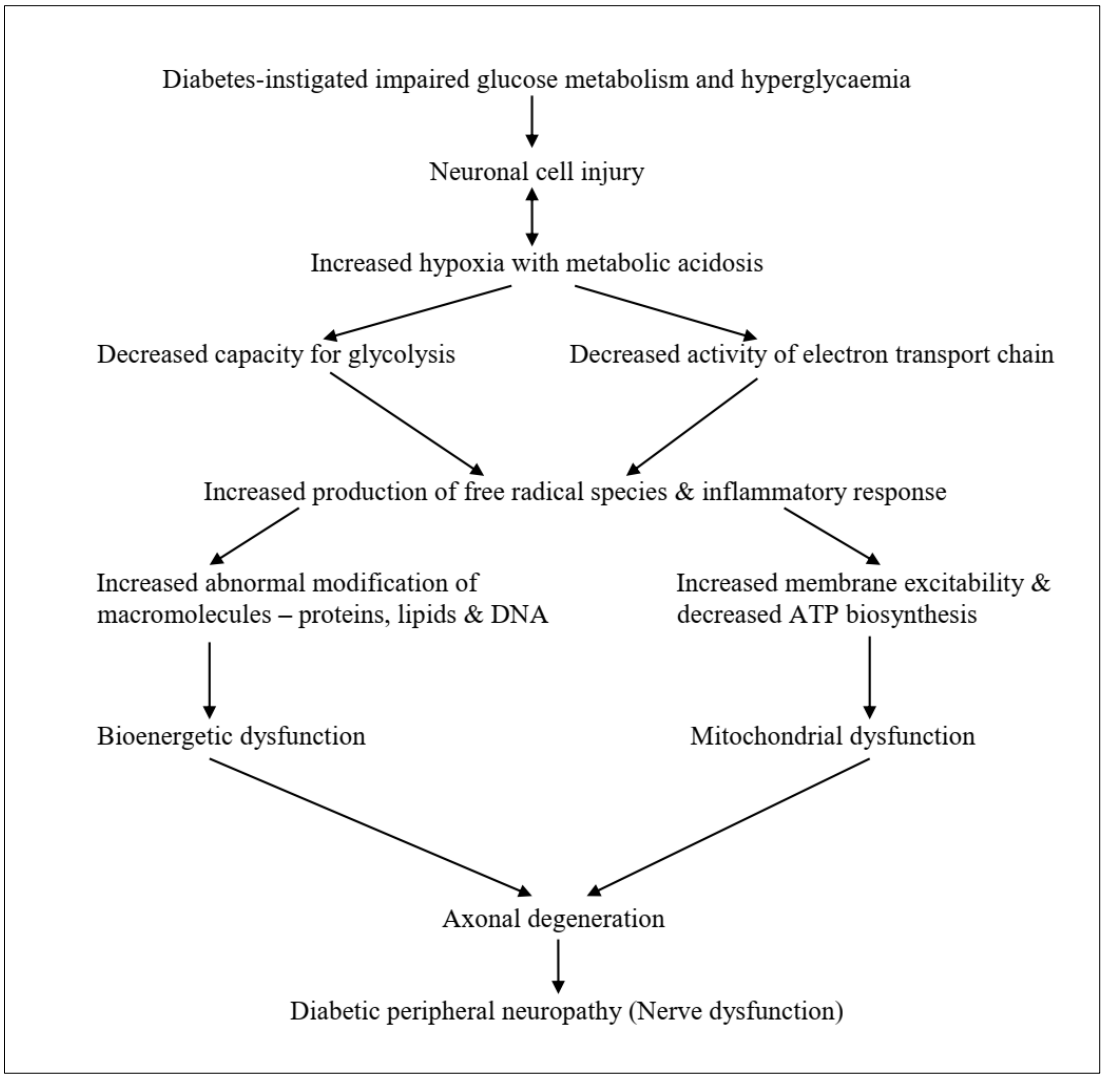

Fig. 3: A Schematic Representation of the Molecular Cascade in Diabetic Neuropathy

These destructive pathways give insights into the detrimental effects of FRS on the cellular and molecular signalling in chronic hyperglycaemia-induced DN.

1. Polyol Pathway:

Glucose metabolism ideally goes through the glycolytic pathway, TCA cycle, and then oxidative phosphorylation (Fernyhough and McGavock, 2014). The excess glucose is biotransformed (or reduced) to sorbitol and fructose by the enzyme, aldose reductase. In excess of glucose, the polyol pathway is activated for glucose metabolism in the peripheral nerves, instead of glucose cascading down the glycolytic pathway. This increases the concentration of sorbitol and fructose, and lowers the expression and uptake of inositol. As a consequence, the activity of Na^+/K^+-ATPase declines (Ott *et al*., 2007), and nerve conduction velocity slows down leading to peripheral nerve damage. NADPH (a synthetase) converts GSSG (oxidised glutathione) to GSH (reduced glutathione). In the reduction of glucose to sorbitol by aldose reductase, NADPH is converted to $NADP^+$, thereby reducing the conversion of GSSH to GSH. Increased levels of sorbitol counter-balances the osmotic equilibrium in the cells culminating in osmotic stress and a compensatory outflow of inositol and taurine. Loss of inositol causes damage to the normal structure and function of the neurones. Excessive activation of the polyol pathway facilitates the development of DN. Sorbitol is converted to fructose by sorbitol dehydrogenase, utilising NAD^+ as cofactor, and both sorbitol and fructose, and other metabolic intermediates promote oxidative stress and neuropathy (Langer *et al*., 2020). Accumulation of sorbitol in the axons and disruption of the Na^+/K^+-ATPase enzyme system promotes early neurological dysfunction (Kobayashi and Zochodne, 2020).

2. Protein Kinase C Pathway:

The glycolytic pathway is a critical pathway in the metabolism of glucose, and glucose metabolism is an important process in PKC and AGE pathways. Glucose is transported into the liver and muscle cells by GLUT-1 and GLUT-3 for glycolysis. In the process, glucose is phosphorylated and metabolised, which produces metabolic intermediates (e.g., glucose-6-phosphate,

fructose-6-phosphate, glyceraldehydes-3-phosphate and pyruvate) (Mizukami and Osonoi, 2020). When diabetes mellitus ensues, glyceraldehydes-3-phosphate is converted to diacylglycerol, which in turn activates the PKC pathway in the nerve cells (Feldman *et al*., 2017). The pyruvate formed during glycolysis enters the mitochondrial TCA (Kreb) cycle and produces NADH and $FADH_2$. The glucose-6-phosphate is then oxidised to produce ATP. PKC activation in turn activates and phosphorylates ATPase leading to metabolic damage and disorders, thereby disrupting normal metabolic process in the body. Activation of PKC also decreases the viability of neuroglial cells and glutamate uptake, and thus increases the formation of FRS which cause oxidative damage. The PKC pathway is touted to be a potential therapeutic target, especially the treatment of axonal injury. PKC inhibitors and activators are currently being considered for the treatment of PKC-mediated disease.

3. Advanced Glycation End Pathway:

Glycation end-products are developed when proteins and fats or other biomolecules (e.g., DNA) are exposed to high concentrations of blood glucose, thereby forming a variety of compounds with high levels of reactivity. Glycation is a reversible-*cum*-irreversible, non-enzymatic sugar-protein covalent bond that results in the formation FRS. Once glycation sets in, it becomes progressive. Examples of glycation end-products are glycohaemoglobin, imadazolone, pentosidine, carboxymethylarginine, etc. The AGE pathway uses AGEs as triggers. AGEs bind to late glycation end-product receptors to activate chemotactic factors and pro-inflammatory markers. The accumulation of AGEs leads to a cascade of inflammatory reactions and causes microvascular damage and glial cell dysfunction. AGEs activate NADPH oxidase and increase the production of FRS. These reactive species generated, in turn, promote the process of AGE production (Ismail-Beigi *et al*., 2010). Overtime, the increased levels of FRS, in turn, heighten irreversible oxidative stress and neuronal cell death (The Action to Control Cardiovascular Risk in Diabetes Study Group, 2008; Calles-Escandon *et al*., 2010).

4. Hexosamine Pathway:

This biochemical pathway, a relatively minor branch of the glycolytic pathway is triggered by hyperglycaemia (Marshall *et al*., 1991; Buse, 2006). This damages the Chevron cells and neurones *via* oxidative stress and inflammation, resulting in diabetic neuropathy. Fructose-6-phosphate is converted to glucosamine-6-phosphate (and serves as a donor of the $R–NH_2$ group) by the first and rate limiting enzyme, glutamine: fructose-6-phosphate aminotransferase. The end-product of the enzymatic reaction is uridine diphosphate-N-acetylglucosamine, a key substrate for protein glycosylation. UDP-N-acetylglucosamine modulates protein activity and expression of pro-inflammatory and pro-coagulant factors. UDP-N-acetylglucosamine binds to amino residues (e.g., serine and threonine), and abnormally modifies transcription process. About 2 - 5 % of the total sugar from glucose metabolism enters the hexosamine biosynthetic pathway *via* fructose-6-phosphate, which increases in dyslipidaemic (hypertryglyceridaemic) stressed cells (Marshall *et al*., 1991).

5. Poly (ADP-ribose) Polymerase Pathway:

Polymerase is a nuclear DNA repair enzyme that generates protein-binding ADP-ribose by splicing NAD^+, which causes energy insufficiency and induces oxidative stress (Shi *et al.*, 1999). The enzyme is associated with decreased nerve conduction velocity, disorders of nerve and blood vessels, mechanical allodynia and thermal hyperalgesia, and loss of myelin sheath (Levine *et al.*, 1999; Shi *et al.*, 1999; Halliwell and Whiteman, 2004). Oxidative stress plays a significant role in the development of DN. Poly (ADP-ribose) polymerase pathway activation, a downstream effector of oxidant-induced DNA, is an obligatory step in functional and metabolic changes in the diabetic nerve (Obrosova *et al.*, 2004). Therefore, the development and application of PARP inhibitors may be helpful in the clinical management of this intractable metabolic disorder. Studies in laboratory models of PARP-deficient mice were protected from both diabetic and galactose-induced motor and sensory nerve conduction slowing and nerve energy failure that were present in mice with intact PARP (Obrosova *et al*., 2004).

6. Mitogen-Activated Protein Kinase Pathway:

DN is linked to the activity of MAPK in the peripheral nerve, and this activation is in response to extracellular stimuli, including tumour necrosis factors (TNF) and interleukins - IL (e.g., TNF-α and IL-1β) (Vieira *et al*., 2022). There appears to be a causal relationship between increased levels of p38-MAPK expression due to pro-inflammatory IL and DN. There are three sub-families/ sub-types of MAPK *viz* extracellular signal-regulated kinases (ERK), c-jun NH_2-terminal kinases (JNK), and p38 kinases. Through regulation of transcription factor activation, MAPKs play a pivotal role in many cellular responses, such as survival, growth, differentiation, maintenance of phenotype, and death (Geilen *et al*., 1996). In diabetic animal-induced oxidative stress, high glucose concentrations activate p38-MAPK to cause cell damage. Activation of all the three sub-types of MAPK by osmotic disequilibrium and increases in the levels of ERK, JNK, and especially p38 in sural nerve specimens of diabetic patients demonstrates the involvement of p38-MAPK in the pathogenesis of DN through direct effects of glucose, glucose-instigated oxidative stress and by advanced glycated end-products *via* its receptors (Purves *et al*., 2001).

PHYSIOPATHOLOGICAL MECHANISMS OF DIABETIC NEUROPATHY

DN is one of the most debilitating outcomes of diabetes mellitus, which can cause pain, reduced mobility, bladder problems, dyslipidaemia and amputation, due to different types of damage to the peripheral nerves (Dewanjee *et al.*, 2018). The pathogenesis of DN is very complex. Diabetes mellitus can damage the PNS in diverse ways. Two hypotheses have been advanced to elucidate the pathology and the mechanistic basis of DN.

1. Vascular Hypothesis:

This theory postulates that occlusion of the *vasa nervorum* (i.e., small arteries that provide blood supply to the peripheral nerves) is the principal cause, and this is plausible in isolated mononeuropathies.

2. Metabolic Hypothesis:

Pertubations in the metabolic pathways caused by excess blood glucose concentrations instigate oxidative stress in the PNS, and thus result in axonal and neuronal injuries. The diffuse symmetrical nature of the common forms of diabetic peripheral neuropathy lends credence to a metabolic origin of the disorder.

Chronic hyperglycaemia causes the formation of sorbitol and fructose in the Schwann cells, and the accumulation of these sugars may disrupt the function and structure of the cells. The earliest functional change in the nerves of individuals with diabetes mellitus is delayed nerve conduction velocity while the earliest histological change is segmental de-myelination caused by damage to the Schwann cells. In the initial stages of DN, the axons are intact (preserved), but with the progression of the condition, irreversible axonal degeneration ensues (Holt, 2021). In diabetes mellitus, mitochondrial integrity, motility and localisation are inimically affected along the axon (Fernyhough and McGavock, 2014; Fernyhough, 2015). The Schwann cells produce acylcarnitines, which induce extracellular Ca^{2+} flux in the axon (Viader *et al.*, 2013). This action hinders axonal mitochondrial trafficking leading to paucity of axonal energy production and mitochondrial apoptosis, as demonstrated in laboratory diabetes (Fernyhough, 2015).

Multiple forms of DN abound, and ranges from discomfort to death. The pathology of DN is characterised by progressive loss of nerve fibre, which produces positive manifestations (i.e., walking on cotton wool, pain, paraesthesia and dysesthesia, increased risk of ulceration) and negative manifestations (i.e., numbness and loss of sensation) (Yagihashi *et al*., 2007). Numbness (hypoesthesia) in the hand or feet is often bilateral, and described as stocking-and-glove distribution. Paraesthesia refers to abnormal sensations such as prickling, tingling, pins and needles, and burning sensation in the feet, while dysesthesia refers to painful feelings (i.e., itching, prickling, crawling, tingling or stinging). The loss of nerve fibre is characteristically a pan-modal pattern with proximo-distal gradient. Endoneurial microangiopathic changes are constant features of peripheral nerve disease, and negatively correlates with nerve fibre density. Progressive loss of nerve fibre involves a multiplicity of factors, including the sorbitol pathway, glycation-induced production of FRS, and changes in the activity of protein kinase C (Yagihashi *et al.*, 2007). Neuronal cell pathology may originate from changes in the structure, electrical and/or its chemical composition. It may also stem from myelination process (i.e., formation of myelin sheath, the protective covering of the neurone), and may lead to defective protection and electrical activity in the neurones. Myelin sheaths are produced by the Schwann cells in the PNS and oligodendrocytes in the CNS through the process of myelination, and any interruptions in the process will cause de-myelination, as seen in certain diseases of de-myelination (e.g., DN and multiple sclerosis). The pathological hallmarks of DN include: micro-angiopathy of the *vasa vasorum* (i.e., small blood vessels that supply blood to the walls of large arteries and veins), axonal loss, axonal atrophy, and de-myelination (Malik, 2014). De-myelination affects impulse conduction in the PNS and transmission in the CNS. This entirely blocks or decreases the velocity of impulse conduction in the affected neurones, which in turn diminishes sensation and other neuronal activities. In de-myelination, impulse does not travel from one node of Ranvier to the next, separated by myelin sheaths. In axons without myelin sheath (i.e., de-myelinated or unmyelinated nerve fibre), the velocity of impulse conduction is proportional to the square root of the axonal inner diameter of the neurone (Bangalore and Waxman, 2012). In physiological state, however, impulse jumps from one node of Ranvier to another, a condition described as saltatory conduction. Damage to the myelin sheath exposes the neurone to harsh and destructive environmental conditions, and may eventually lead to the death of some of the neurones. Even though, adaptation may take place in the remaining neurones, the ultimate result of de-myelination is DN. DN if not promptly addressed and allowed to progress and fester, may lead to limb amputation or even death, thus reducing the life expectancy of the patient due to compromised cardiovascular functions modulated by the autonomic nervous system. These conditions result from tissue damage, which are common occurrences in long-term diabetic complications.

PHARMACOLOGICAL TREATMENT OF DIABETIC NEUROPATHY

Very few therapeutic modalities are available that can contain the cause of the illness. There is, however, no definitive treatment of the degenerative disorder and prophylaxis against DN is non-existent. Achieving normoglycaemia using anti-diabetic regimens and foot-care is critical to any therapeutic objectives. The goal of therapy, therefore, is to delay the onset of DN and prevent its complications, thereby reducing the severity

among the sufferers and improving the quality of their lives. Treatment of DN is broadly divided into two, pharmacological and non-pharmacological.

A. **Pharmacological Approach:** The current pharmacotherapeutic approaches for DN is a dual concept based on their action. These include**:**
   1. Aetiology-based treatment: This involves the application of therapeutic agents to target pathogenesis of the disorder. The objective of the therapy is to stabilise glycaemic status by achieving euglycaemia using antidiabetic (hypoglycaemic) medications (i.e., insulin and oral hypoglycaemic agents).
   2. Treatment of risk factors: This is the foundation of therapy, and helps to ameliorate the clinical symptoms found in DN.

A variety of medicaments are clinically employed for the treatment of DN. These agents are:

1. Lipoic/ Thioctic Acid (Acetate Replacing Factor - ARF):

ARF is a congener of vitamin B complex, an antioxidant that is endogenously produced. It is present in certain foods (e.g., carrots, tomatoes, potatoes, beets, yeast, spinach, green peas, organ meats – heart, liver, kidneys, spleen, etc.). Lipoic acid is an aetiology-based therapy (Bril *et al*., 2009; Fraser *et al*., 2012 a,b; Mijnhout *et al*., 2012) and plays a key role in mopping up FRS that speed-up ageing process and cause diseases. It reduces oxidative stress through the inhibition of HBP and AGEs pathways. Alpha-lipoic acid has demonstrated some promise in short-term therapy for DN, particularly the insulin resistance (Mahmoudi-Nezhad *et al*., 2021). Lipoic acid has also shown some promise in several human experimental trials (Ametov *et al*., 2003; Ziegler *et al*., 2006; Papanas and Ziegler, 2014). However, long-term therapeutic significance of α-lipoic acid (ALA) is lacking. It can be used singly or combination with other agents. Combination therapy using ALA with superoxide dismutase has improved symptoms and electroneurographic parameters in patients with DN (Bertolotto and Massone, 2012). Examples of ALA, include benfotiamine (a pro-drug of thiamine monophosphate; a lipid and water soluble derivative of thiamine; a vit. B_1 analogue). Benfotiamine blocks diabetes-induced activation of the HBP, AGE and PKC pathways (Brownlee, 2001; Brownlee, 2005), and in so doing, prevents the formation and accumulation of AGEs. Benfotiamine has demonstrated both preclinical (Brownlee, 2005; Sanchez-Ramirez *et al*., 2006) and clinical efficacies in DN (Winkler *et al*., 1999; Haupt *et al*., 2005; Stracke *et al*., 2008).

2. Aldose Reductase Inhibitors (ARIS):

ARIs are monocarboxylic acid (─COOH) derivative that non-competitively and reversibly inhibits aldose reductase, an enzyme that biocatalyses the conversion (reduction) of glucose to sorbitol in the polyol pathway, and thereby diminish the reduction in nerve conduction velocity, and so, sorbitol has been linked to DN (Yagihashi *et al*., 2001; Caramori *et al*., 2003). ARIs target specific oxidative stress pathway, and reduce glucose flux into the polyol pathway. This facilitates nerve conduction velocity in the sural motor and sensory nerves, improves wrist and ankle F-wave latency (i.e., time it takes for electrical impulses to travel from the site of nerve stimulation to the recording site or device; the amplitude of the response is diagnostic of peripheral neuropathy), and alleviating neuropathic pain (Yagihashi *et al*., 2001). Epalrestat decreases intracellular sorbitol accumulation, which has been implicated in the aetiopathogenesis of late-onset complications of type II Dm, including DN. It is touted to be highly effective and safe in treating DN, although long-term clinical benefit is yet to be demonstrated (Bril *et al*., 2009; Fraser *et al*., 2012 a,b; Mijnhout *et al*., 2012). Aldose reductase inhibitors, include epalrestat, ranirestat, zopolrestat, minalrestat, lidorestat, sorbinil, etc.

3. Antiseizure Drugs:

Pregabalin and gabapentin ($\alpha_2\delta$ ligands) are ϒ-aminobutyric acid analogues and the first-line agents in the treatment of painful diabetic neuropathy. Pregabalin is preferred to gabapentin, because the onset of pain relief is faster, and should, therefore, be used to initiate treatment in DN. Adverse effects may include drowsiness, dizziness and swelling of the hands and feet. As a result, caution must be exercised during driving and those operating machineries. Carbamazepine can also be used (as a third-line agent) if the other two medicaments fail to provide the desired therapeutic effect.

4. Antidepressants:

Agents used in clinical depression have been used to treat DN [e.g., amitriptyline and desipramine (TCAs – tricyclic antidepressants), duloxetine and venlafaxine (SNRIs – serotonin-noradrenaline re-uptake inhibitors)]. Amitryptyline and desipramine are reasonable first-line TCAs while duloxetine is recommended as first-line antidepressant treatment for DN followed by venlafaxine. These agents have fewer tolerable side effects.

5. Opioids:

Opioid analgesics (e.g., morphine, methadone, tapentadol, and tramadol) are recommended either as second or third-line agent for the relief of symptoms of DN for a short duration.

6. NSAIDs and Acetaminophen:

Both agents may be used as first-line therapy to relieve pain in patients with acute painful conditions due to diabetes-induced peripheral nerve damage.

7. Prostaglandin E_1:

PGE_1 (e.g., alprostadil) relaxes the blood vessels, decreases viscosity of the blood, and inhibits

plate aggregation, and in so doing, improves the microcirculation. It is employed with other agents for better clinical benefits. For instance, it can be combined with α-lipoic acid or vit. B_{12} (methylcobalamine) for greater clinical efficacy. Combination of gabapentin with vit. B_1 – B_{12} produces a synergism due to their anti-allodynic and anti-hyperalgesic effects, and thus significantly reduces pain in patients with DN.

8. Acetyl-L-Carnitine (ALC):

This agent is an endogenously derived nutrient, and is available as supplement. ALC may assuage nerve pain in certain individuals and principally targets FRS.

9. N-Acetyl-Cysteine (NAC):

NAC is a cysteine prodrug and glutathione precursor. It targets FRS, preventing oxidative stress and cellular damage. It has demonstrable capacity to retard progression in DN through its neuroprotective, antioxidant, and antiinflammatory activities (Grinberg *et al*., 2005). NAC can improve insulin resistance, thereby regulating the blood glucose concentrations.

10. ACE Inhibitors:

Trandolapril is an ACE inhibitor employed in the therapy of DN. The agent may improve the symptoms of peripheral neuropathy in normotensive patients with background diabetes mellitus. Large scale clinical (human) experimentation is still needed for its confirmation and advocacy.

11. Botulinium Toxin:

Botulinium toxin, also referred to as *miracle poison* is one of the most poisonous biological substances known to humans. It is a neurotoxin produced by *Clostridium botulinium*, a gram-positive, spore forming, anaerobic rod. Intra-dermal injection of botulinium toxin type-A for the treatment of pain of DN is relatively novel. It has a long-lasting (up to 3-months) pain control with minimal side effects (Bayat *et al*., 2023). However, significant improvement in the quality of sleep, physical or mental function is doubtful. Botulinium toxin type-A controls pain *via* the inhibition of release of inflammatory mediators (e.g., calcitonin gene-related peptide).

12. Topical Agents:

A topical agent used in DN is 8 % capsaicin patch or 0.1 % capsaicin cream. Transdermal capsaicin patch and capsaicin cream have both demonstrated efficacy in pain relief, especially numbness and tingling sensation. However, transdermal capsaicin patch contains higher concentrations of capsaicin than the cream. Capsaicin is the substance found in 'chilli' pepper. It improves pain and the quality of life. Side effects may include burning sensation and skin irritation. Isosorbide dinitrate (a nitro-dilator and anti-anginal medication) spray is employed in the treatment of pain and burning sensation arising from DN. Local anaesthetic agent such as 5 % lignocaine (lidocaine) patch can significantly provide relief for intractable neuropathic pain up to a period of 3-weeks.

B. **Non-Pharmacological Approach:** Non-drug measures for painful DN are adjuncts to pharmacotherapy. They include the following:

1. Psychological/ Emotional Support:

This form of therapy provides the mental, emotional, social and spiritual needs of the patient and those of the family while providing reassurance and listening, and without being unnecessarily judgemental.

2. **Acupuncture**: This is a form of alternative/ complementary medicine. It may relieve pain of DN and is touted to have no side effects.

3. Physiotherapy:

Physical therapy prevents and relieves pain, decreases inflammation, calms the nerves, improves range of motion/movement, restores body function, and delays symptoms of degenerative illness.

4. Transcutaneous Electrical Nerve or Muscle Stimulation (TENS):

This procedure blocks pain signal transmission to the CNS. This is achieved by delivering tiny electrical impulses to specific neuronal pathways *via* small electrodes placed on the skin. It is painless and safe, but it does not work for all patients neither does it effectively address all types of pain in DN.

5. Spinal Cord Stimulation:

This modality of treatment is employed in patients with painful DN that are unresponsive/pharmaco-resistant/pharmaco-refractory to the best available treatment. High frequency (10-kHz) spinal cord stimulation improves outcomes in patients with refractory painful DN up to a period of 6-months (Petersen *et al*., 2021).

CONCLUSIONS

Diabetic neuropathy is the commonest long-term neuromuscular complications of diabetes mellitus. It is caused by poorly controlled or uncontrolled diabetes mellitus with consistent elevated blood glucose concentrations. Oxidative stress arising from hyperglycaemia is the most universally accepted cellular and molecular underpinnings for the development and progression of DN. Damage to the myelin sheath exposes the neurone to harsh and destructive environmental conditions provoked by chronic hyperglycaemia with oxidative stress, and may eventually lead to death of some of the neurones. DN relatively manifests early, affecting a significant proportion of the micro-blood vessels in the middle aged and elderly diabetic patients. Chronic elevated blood glucose concentrations cause the activation and inhibition of several biochemical pathways that are crucial for homeostasis in neuronal and neuroglial cells. DN if not promptly addressed and allowed to progress and fester, may lead to amputation

or even death, thus reducing the life expectancy of the patient due to compromised cardiovascular functions modulated by the autonomic nervous system. Despite the plethora of molecular research activities into DN, there is still no known definitive therapy. This is because of the discordance between basic (preclinical) studies and clinical (human experimental) trials in patients with DN. Treatment at the moment is essentially symptomatic, with huge economic and psychological burden, both to the sufferer and the family alike. The goal of therapy, therefore, is to slow the progression of the disease, relief pain, manage complications, and restore or normalise body function, including neuropathy-related pathological sequelae. In addition, lifestyle adjustments in obese and overweight individuals with diabetes mellitus, including weight loss is a *sine qua non*, and loss of 5 – 7 % of total body weight with moderate physical activity for at least 2.5 h weekly is ideal, since pharmacotherapy alone is not sufficiently adequate to combat and contain the disorder.

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