International Journal of Healthcare Sciences ISSN 2348-5728 (Online)
Vol. 8, Issue 2, pp: (261-276), Month: October 2020 - March 2021, Available at: www.researchpublish.com

Pharmacological approach for the treatment of diabetic neuropathic pain: A Control of discomfort using gene therapy

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Abstract: **Diabetes Mellitus is one of the most common diseases in the world, affecting millions of peoples every year, one of its most serious complications is diabetic neuropathy and the associated neuropathic pain resulting from various causes within the body of a diabetic patient, most commonly it is the nerve damage that causes this pain due to over accumulation of glucose in the body, but this is not the sole reason for pain generation. In this review article, different factors causing the pain in diabetic patients is discussed alongside with pathogenesis of diabetic neuropathy to understand the mechanism of pain generation and it treatment options based on gene therapy techniques dealing with pain generation and sensation on cellular and molecular basis. Gene therapy is a modern revolutionary technique aiming at treating the cause of the pain rather than masking the pain sensation like conventional therapy techniques do, however, gene therapy is a novel technique which is still not fully developed and still have a number of challenges and limitations regarding its safety, effectiveness and cost.**

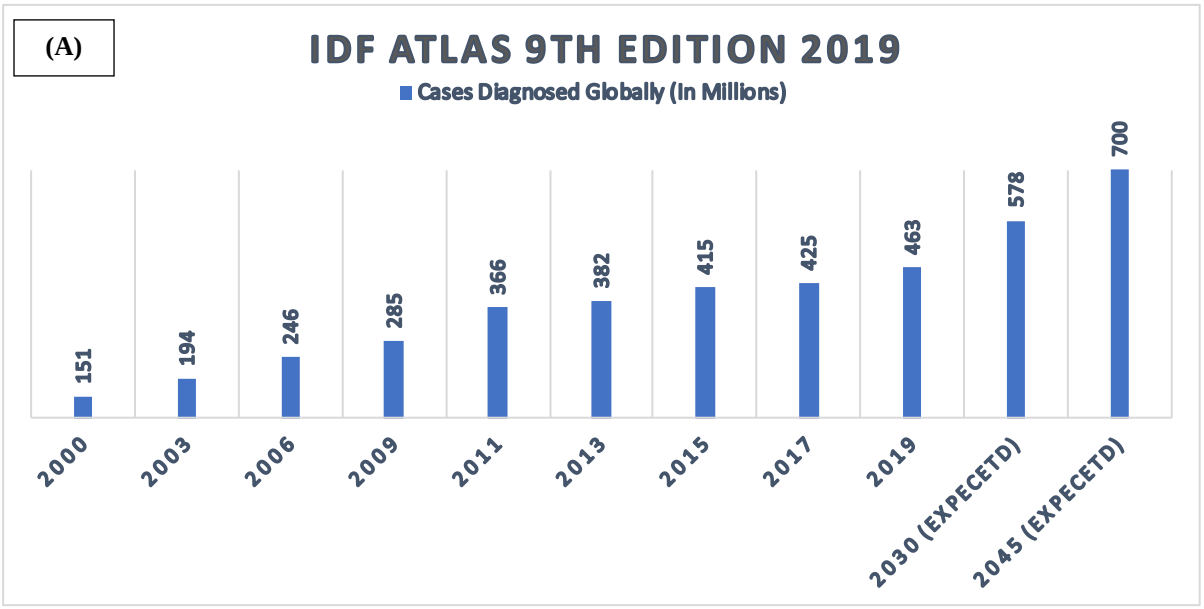
Keywords: **Neuropathic Pain, Gene Therapy, Diabetes Mellitus, Diabetic Neuropathy.**

1. INTRODUCTION

1.1 Diabetes Mellitus

Diabetes mellitus, commonly known as “diabetes”, is a chronic noncommunicable prevalence disease globally, occurring when there is inadequate production of insulin from the pancreas (Type 1) or the inability of the body to utilize the produced insulin effectively (Type 2), uncontrolled diabetes can lead to accumulation of glucose in the blood, elevating it levels, creating a status of “hyperglycaemia” causing multiple hazards to the health and well-being of the patient, including damage to the blood vessels, nerves and other vital organs.[1]

Diabetes mellitus has been increasing at an alarming rate, as showing in the below charts, according to the International Diabetes Federation (IDF) database, there has been more than 205% increase in diagnosed cases from 2000 to 2019, with further increase expected in the upcoming years.[2]



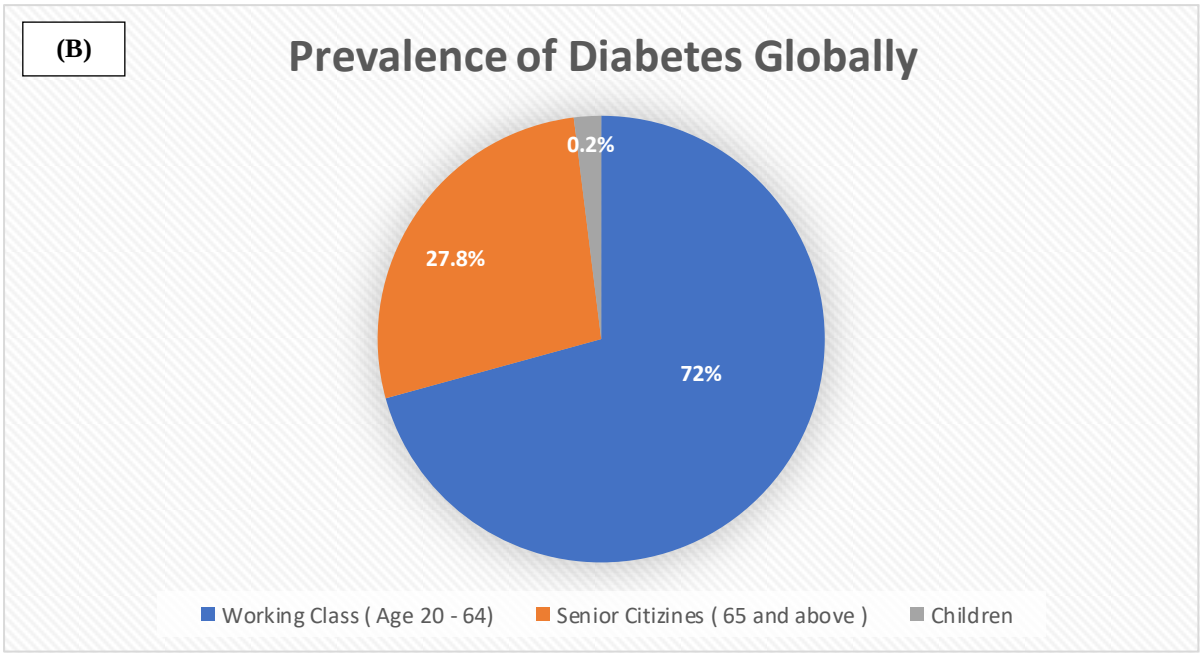


Figure (1): (A) Global increase in diabetes mellitus cases between 2000-2019, (B) Prevalence of Diabetes Globally.

1.2 Neuropathic Pain

Neuropathic pain is a condition affecting many patients around the world, it is basically a condition where there is an injury or damage to the nerves throughout a patient's body, since these nerves are the main transportation and communication tools of the human body, resembling a huge network that runs through the human body connecting it to the controlling centres represented by the brain and the spinal cord, damage in any part of this network can cause discomfort, pain and other complications depending on the type and position of the injured or damaged nerve, thus it can result in symptoms ranging from mild, discomforting and up to disabling and fatal, general symptoms includes feeling of pain, burn and hypersensitivity in affected areas, numbness and fatigue alongside with muscle crumps.[3]

1.3 Gene Therapy

Gene therapy is applied to either replace a dysfunctional gene, introducing a new gene into the body to help fight a disease or production of a new useful cell components such as (proteins ,missing factors ..etc) to restore the patient's health and well-being or provide relief of certain discomforts or pain sensations throughout the body.[4]

It is a revolutionary technology that provides a new approach in treatment and relief of complications, it aims at targeting the cause of disease or discomfort at molecular basis, thus providing long term relief or in some cases, permanent cure for a number of usually chronic and untreatable conditions by the conventional routes of therapy.

2. DIABETIC NEUROPATHY / NEUROPATHIC PAIN

2.1 Pathophysiology

2.1.1 Types and Aetiology

One of the most serious complications of diabetes mellitus is the diabetic neuropathy, it is when high blood glucose level cause damage to different nerves throughout the patient's body, causing both pain and other different conditions depending on the type and position of nerve injured, it is a very common complication and occurs in variety of types affecting up to 66% of type 1 and 59% of type 2 diabetes patients, the symptoms range from mild to painful and its critical status ranges from discomforting, disabling and up to fatal.[5]

Diabetic neuropathy have 4 main types as illustrated in the figure below, differ from each other in the areas affected, but have similar signs and symptoms such as (Hyperesthesia and hypoesthesia, impaired coordination, pain, pins and needles sensation, muscle weakness or wasting, bloating, nausea, indigestion, vomiting, diarrhoea or constipation, dizziness, hyperhidrosis or anhidrosis, urinary retention, vaginal dryness, erectile dysfunction, diplopia, and tachycardia).[6] There are other causes than hyperglycaemia such as, dyslipidaemia, impaired insulin signalling, growth factor deficiency, vascular deficiency, neurovascular interaction or metabolic disorder syndromes.

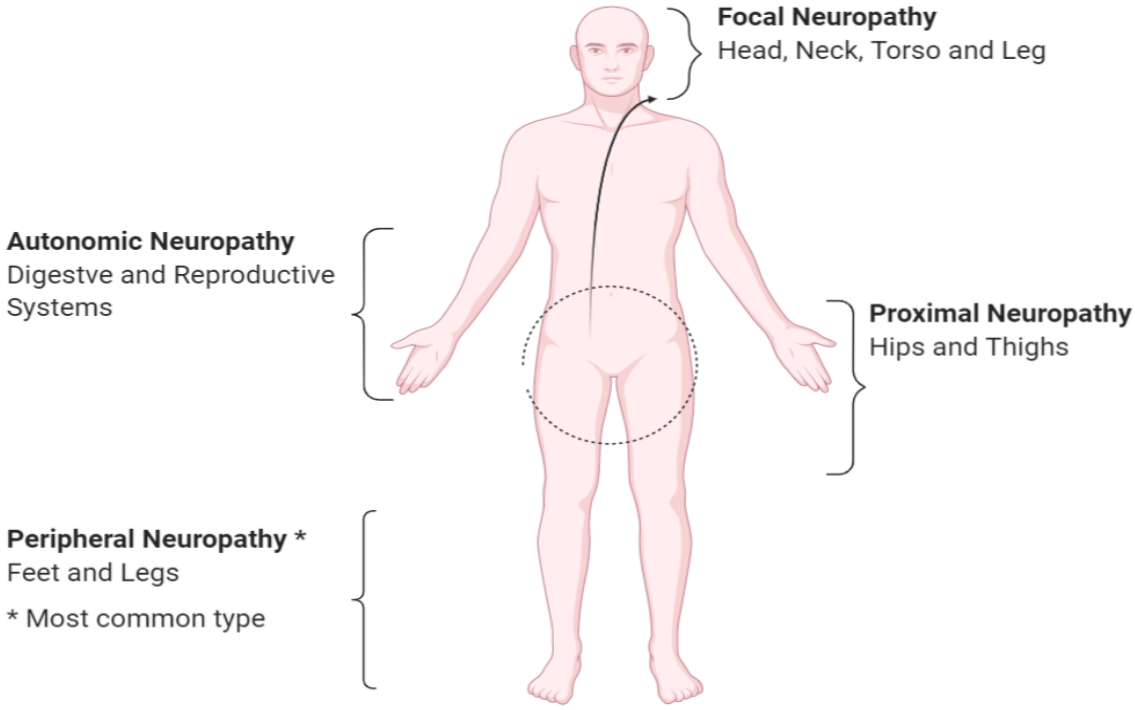


Figure (2): Types of Diabetic Neuropathies and Affected Areas

In case of diabetic neuropathy, although scientists have not yet proven the exact reason or cause for each type of diabetic neuropathy, they were able to lay down the most acceptable pathway for its occurring, and narrowed down the risk factors into a few possible ones, the most acceptable pathway according to researchers is that the uncontrolled elevated levels of glucose in the blood for prolonged time periods causes physical damage to the nerves, interfere with its signalling process and cause interruptions in the blood capillaries supplying nutrients and oxygen to the nerves.[7] Risk factors on the other hand can be shortlisted as per the following list, uncontrolled / unmonitored blood sugar levels, diabetes history in the family, unhealthy diet, sedentary lifestyle and lack of exercise, consuming tobacco and alcohol, elevated levels of low-density lipoprotein (LDLs) and low levels of high-density lipoprotein (HDLs), cardiac and blood vessels disorders and renal and hepatic disorders.[8]

2.1.2 Pathogenesis

From the figure above, different process involved in pathogenesis of diabetic neuropathy is noticed,

1. Oxidative stress, whereas oxidation of different biomolecules and inflammation have an important role in promoting different conditions having harmful effects on cellular components, oxidative stress is known to have a wide range of damaging effects in the body due to over production of reactive oxygen species or lack of anti-oxidants, it can cause the physical damage directly or by initiating an inflammatory response which eventually cause the damage through different direct and indirect mechanisms, it is also mentioned that oxidative stress was found in a recent study having probability to be induced by psychological stress, thus it can be possible that emotional stress can also be risky for diabetic patients.[9]

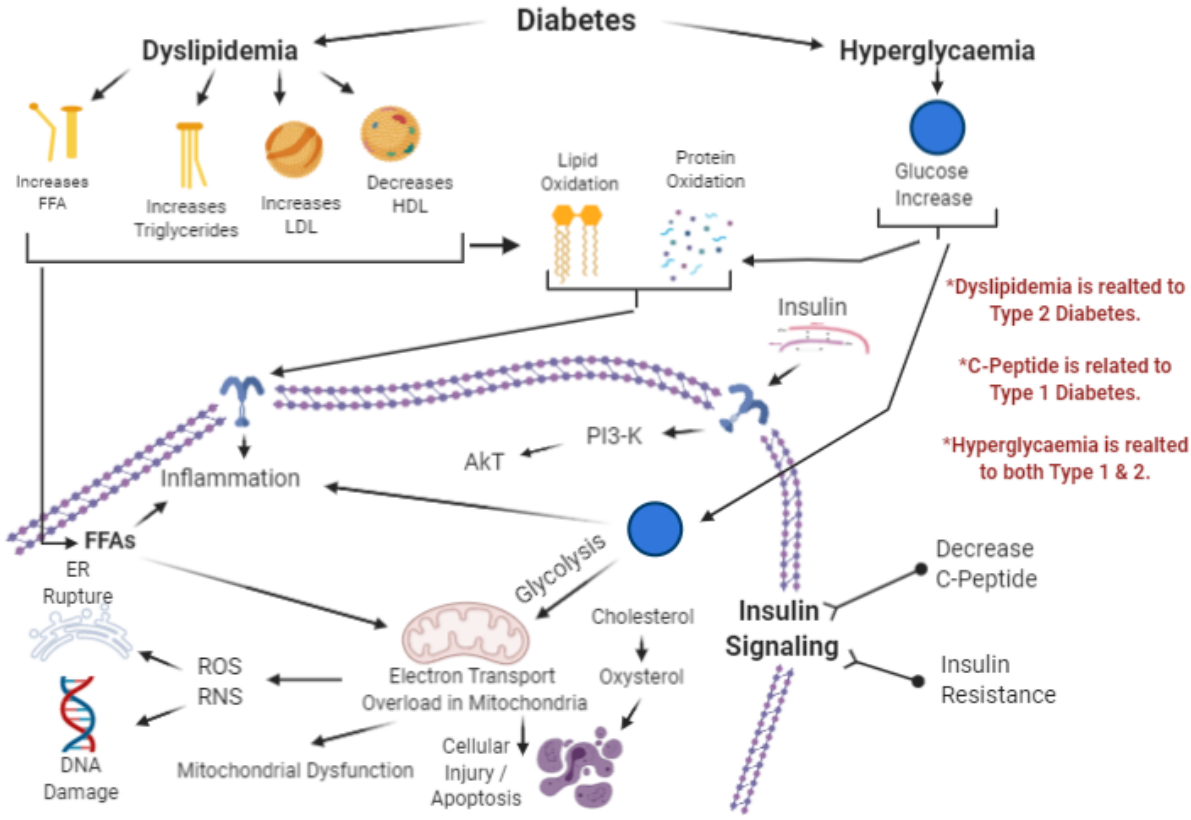


Figure (3): Pathogenesis of Diabetic Neuropathy

FFA: Free Fatty Acids. PI3-K: Phosphatidylinositol-3 Kinase. ER: Endoplasmic Reticulum. AkT: Protein kinase B. ROS: Reactive Oxygen Species. RNS: Reactive Nitrogen Species.

2. Oxidation of free fatty acids is linked to many diseases and disorders such as metabolic syndrome, atherosclerosis, diabetes mellites and others, causes disruption in the cell membrane and mitochondrial dysfunction due to overload in the electron transport chain of the mitochondria, since the mitochondria is the powerhouse of the cell, damaging it will also cause cell injury, inadequate energy production, and might reach up to death of the cell.[10]

3. Oxidation of protein yields protein carbonyl contents, which is found to be higher in diabetes patients in comparison with healthy people, suggesting a relationship between presence of these contents and diabetes therefore with diabetic neuropathy as well.[11]

4. Oxidation of cholesterol results in oxysterol, having damaging effects on the central nervous system (CNS), leading to many CNS diseases and disorders and found to be in high concentrations in the blood samples of diabetes patients.[12]

5. Insulin role, insulin regulates many metabolism processes through PI3K/AKT signalling pathway which initiate lipid biosynthesis and inhibits lipolysis, any obstacles in this pathway have multiple hazards on different processes of the body including those related to diabetes and diabetic neuropathic complications.[13]

6. Glucose regulation, when glucose level increases in the body it promotes polyol pathway activity, which converts glucose to sorbitol by the action of enzyme aldose reductase with oxidation of nicotinamide adenine dinucleotide phosphate NADPH to NADP+, sorbitol is then oxidized into fructose by sorbitol dehydrogenase, coupled with the reduction of nicotinamide adenine dinucleotide NAD+ to NADH, during hyperglycaemic state, aldose reductase affinity for glucose is high, excess sorbitol generates intracellular osmotic stress due to accumulation of sorbitol, this can explain why some patients develop sensation of pain, but it is a plausible pathway and not a proven fact yet as it does not explain why some patients develop pain without intracellular accumulation of sorbitol, glycolysis on the other hand regulates glucose production and stimulates insulin secretion, imbalance in this process causes multiple disruption in cell normal behaviour including inflammation and mitochondrial dysfunction.[14]

Both glucose and free fatty acids can cause inflammatory response, however hyperglycaemia can cause this response only when activated by previously existing pro-inflammatory stimuli, such as the cytokine interleukin (IL)1β, while the adipose tissues containing and releasing fatty acids secretes both anti and pro-inflammatory adipokines, free fatty acids also cause insulin resistance both peripheral and hepatic, allowing elevating glucose levels and its consequences.[15]

7. Effects on mitochondria, mitochondrial dysfunction and its related electron transport overload occurring due to different reasons mainly linked to free fatty acids and glucose, their oxidation and inflammation is a serious condition causing cell irreversible injury, death and production of reactive species causing damage to vital cell components such as endoplasmic reticulum and DNA, also with the mitochondria being the power generator of the cell, so any disturbance in it can lead to reduced energy supply for the cell and then its death.[16]

8. In some patients, the abnormal levels of the connecting peptide (C-Peptide), which is a polypeptide consisting of 31 amino acid polypeptides connecting insulin's A-chain to its B-chain, can represent a number of diabetes and non-diabetes related complications, for example, a high level of C-Peptide means insulin is not being utilized in the body as it should be, or the presence of a rare pancreatic tumour known as insulinoma or excess use of sulfonylureas to treat type 2 diabetes, while a low C-Peptide level means either presence of type 2 diabetes, or in case of use of insulin injections then as a result the pancreas stopped production of insulin as the blood glucose level was already low, or in case of insulinoma, then the treatment may had led to the shrinkage of the tumour.[17]

When the injury of the cell occurs in nerve cells it is likely for the patient to develop different kinds of undesirable neurological complications, however, in a number of cases the patients are developing different types of neurological complications without cell or nerve injury, which shows that the exact pathogenesis of diabetic neuropathic pain is still not fully understood, but the cell injury hypothesis is the most convenient and acceptable one so far.[18]

2.1.3 Signs and Symptoms

In addition to previously mentioned common signs and symptoms of types of diabetic neuropathy, there are some symptoms that are characterizing of a certain type or occurring more in a certain type than the others.

1. Peripheral neuropathy, it is the most common type of diabetic neuropathy affecting limbs, targeting feet and legs primarily followed by hands and arms, the signs and symptoms of peripheral neuropathy include, reduced or loss of sensation of pain or temperature variations, especially in the feet and toes, burning sensation, sharp and shocking pain that may be worse at night, hypersensitivity, muscle weakness, loss of reflex response, ulcers, infections, deformities in bone and joint pain in lower limbs[19]

2. Autonomic neuropathy, as the name indicates it affects the autonomic nervous system area and organs related and controlled by this system, such as heart, bladder, lungs, stomach, intestines, sex organs and eyes, possibly causing, hypoglycaemia unawareness, urinary tract infections, urinary incontinence or urinary retention, constipation, diarrhoea, gastroparesis causing nausea, vomiting, sensation of fullness and loss of appetite, dysphagia, erectile dysfunction, vaginal dryness, hyperhidrosis, hyperhidrosis, orthostatic hypotension, body temperature variation, visual adjustment issues with changing light conditions and tachycardia.[20]

3. Proximal neuropathy Also known as (Diabetic Polyradiculopathy or Diabetic Amyotrophy), affects nerves in the thighs, hips, torso or legs, it is more common in people who have type 2 diabetes and in older adults, affecting one body side usually, causes sudden, severe pain in hip and thigh or torso region, weakness and shrinking of the thigh muscles and difficulty rising from a sitting position.[21]

4. Focal neuropathy, known as (Mononeuropathy), usually include damage to a specific nerve, may be in the face, torso or leg, comes suddenly without pervious symptoms, its most common in senior patients, although it can cause severe pain, it usually doesn't cause any long-term problems as symptoms usually disappear on their own over time, depending on which nerve is affected symptoms can be diplopia, paralysis on one side of the face (Bell's palsy), pain in shinbone or foot, pain in the frontal side of thigh, and pain in chest or stomach.[22]

These are the signs and symptoms associated with different types of diabetic neuropathy, as they are somewhat similar with each other and with other diseases as well, it is not common for the patients to seek medical care and tests for diabetes upon feeling one or more of these symptoms, and they can relate these symptoms to lifestyle or stress and pressure of everyday life, thus the diabetes and diabetic neuropathy develops in the patient without being monitored or kept under control, this is why awareness campaigns and early detection and diagnosis is very important.

2.2 Reported Complications in Diabetic Neuropathy[23]

Diabetic Neuropathy has several serious complications that can have a significant effect on a patient's lifestyle and psychological as well as physical well-being, most common ones are,

1. Loss of Limb, in case of peripheral neuropathy, as loss of sensation usually is a symptom of this type of neuropathy, open wounds or sores can go unnoticed until it reach infected or ulcerated level, ulceration causes the soft tissue and skin to break down and high blood glucose level damages the blood vessels reducing the blood supply to the limb, if left untreated, infection can spread to bone and gangrene can occur, gangrene is tissue death which may have no solution but amputation of affected organ or limb.

2.Sexual dysfunction, autonomic neuropathy affects the sexual performance of patients as a result of injured nerves controlling the sex organs, resulting in erectile dysfunction for men while women might experience difficulties with arousal, lubrication, or orgasm.

3. Digestive issues, autonomic neuropathy injures the nerves in the digestive system causing multiple digestive disorders that have severe impact on the patient's lifestyle as it forces a certain diet and may prevent them for certain nutrients as there might be some intolerance and incompatibility issues in the digestive system.

4. Hypotension, as damage to blood vessels and heart is common in autonomic neuropathy, the ability to adjust and monitor the blood pressure and heart rate is compromised, causing the patient to have risk full drops in the blood pressure and heart rate.

5. Pain, one of the most common complications of different types of neuropathy is the pain or discomfort associated with it, drastically affecting the patients as they have to deal with continuous, sometimes severe pain and discomfort which is a very inconvenient condition for them as it is not manageable by conventional therapeutics.

6. Urinary incontinence, this occurs in the case of bladder dysfunction where there is either a partial or total loss of control of the bladder leading to uncontrollable urination, which drastically affects the patient's lifestyle.

3. GENE THERAPY IN DIABETIC NEUROPATHY (WITH FOCUS ON PAIN MANAGEMENT)

3.1 Foundation of Gene Therapy in Diabetic Neuropathy

As diabetes mellitus and its related neuropathy have been considered untreatable by conventional therapeutics, thus there was a need to develop novel approaches to at least reduce the severity of the complications and pain associated with diabetic neuropathy, and thus researchers started to think about molecular basis of the disease and how to prevent these complications by preventing different cellular processes leading to them.

Different techniques have been developed in this field from recombinant DNA technology to produce artificial insulin for those patients who are having inadequate production of it, to adeno associated virus (AAV) and other genetic material carrier techniques which are engineered to work as a vector for genetic and DNA materials to be delivered into target cells up to new nanotechnology based gene therapeutics both organic (viral) and inorganic (non-viral) ones, which will be discussed in the upcoming parts one by one. Before discussing various therapeutics and techniques applied in gene therapy of diabetic neuropathic pain, there is a need to understand how does this pain exactly generates, so that it is possible to manage it by controlling different cellular processes leading to generation of this pain. As previously mentioned in the pathogenies of diabetic neuropathy, insulin regulation disturbances as well as reactive oxygen and

nitrogen species have significant roles in damaging nerves and thus causing pain, alongside these two factors, other factors leading to neuropathic pain are listed in the table below.

TABLE I: Different Factors Causing Neuropathic Pain Schreiber, Anne K (2015). Diabetic neuropathic pain: Physiopathology and treatment. *World Journal of Diabetes*, 6(3), pp. 432-437)

Factor	Biological Events and Effects on Neuropathic Pain
1. Polyol pathway hyperactivity	NADPH and NAD+ levels increase Decrease in glutathione levels Increase in advanced glycation end products (AGEs) Activation of diacylglycerol Activation of protein kinase C (PKC) isoforms Result in oxidative stress and nerve injury causing pain
2. Vascular changes	Reduction of peripheral perfusion in the nervous tissue and the skin0 Increases in vessel wall thickness Hyalinization of the basal lamina Result in nerve ischemia and nerve loss causing pain
3. Nerve endings damage	Disturbance in action potentials, interpreted by central nervous system as dysesthesia or pain Alternations in ion channel expression, leads to hyperexcitability, known to have link with pain sensation Increase number of receptors of voltage-gated sodium channels (Nav), leads to variations in generation and transmission of action potential Result in disturbances in the electrical activity of neurons causing pain sensation
4. Microglial activation	Release of inflammatory mediators and free radicals, damaging nerves and leading to pain Result in spinal sensitization, causing hyperexcitability leading to amplification of painful inflammatory response
5. Spinal cord changes	Enhance Cyclic adenosine monophosphate (cAMP) response Increases glutamate release from the primary afferents Increase N-Methyl-D-aspartate (NMDA) receptor expression Decrease number of receptors gamma aminobutyric acid (GABA) Result in central sensitization, causing hyperexcitability associated with pain
6. Brain changes	Increased peripheral input Increase activity of primary afferents Alternations in pain processing areas such as rostroventromedial medulla (RVM), cortex and thalamus Reduction in levels of N-acetyl-aspartate (NAA) levels Result in central sensitization, causing hyperexcitability associated with pain
7. Peripheral nerves damage	Demyelination of axon Degeneration of neuron Result in polyneuropathy, causing disturbance in signal flow and generalized sensation of pain

These are the most common causes of diabetic neuropathic pain and their related biological events causing pain sensation, although they do not explain the reason behind every painful neuropathic case, however, they do represent the majority of the cases and the most accepted and followed pathway till now, the rest of the cases where the pain occurs without the presence of any factors mentioned in the previous table.

Gene therapy-based treatment for diabetic neuropathic pain has been designed according to the current knowledge of this pain's known aetiology and pathogenesis pathways, and still under research and experiments to determine the safety and potency in diabetic neuropathic pain management, they can either be to reduce the pain and discomfort sensation or to treat the cause of this pain directly on molecular basis, but this one despite its promising primary results, it is still at very

early stages of studies to determine its true potential and efficacy in terminating neuropathic pain of diabetes patients completely, in the coming parts, these different methods, techniques and agents will be discussed.

3.2 Techniques of Gene Therapy Utilized in Diabetic Neuropathic Pain Management

These techniques and agents can be either the ones which do not control or manage diabetic neuropathic pain, but control, regulate and manage different components and/or process that if found in imbalanced or disturbed status will lead to diabetic neuropathy and its related complications including pain, thus becoming indirectly related with pain management or can be directly involved in reducing and relief of discomfort and pain sensation.

3.2.1 Recombinant DNA

As one of the main reasons of diabetes mellitus then its related diabetic neuropathy is the inadequate or ineffective production of insulin, there was a need to provide an alternative preparation from an external source to replace the present insulin, until the early 1980s animal insulin was being used, either porcine or bovine pancreases because animal insulin was somewhat similar in chemistry to human insulin, but still had a lot of side effects of anti-body defensive mechanisms, inactivation and inflammation response until recombinant human insulin preparation was made.

TABLE II: Some Available Insulin Analogs

Cleveland Clinic. 2018. Injectable Insulin Medications. [online] Available at: <https://my.clevelandclinic.org/health/drugs/13902-injectable-insulin-medications> [Accessed 17 October 2020].

S.NO	Analog	Trade Name	Onset of Action	Duration of Action	Developed By	Remarks
1.	Insulin Lispro	Humalog®	10-15 minutes	3-5 hours	Eli Lilly and Company.	May cause Hypoglycaemia, Nasopharyngitis
2.	Insulin Aspart	Novolog®	10-15 minutes	3-5 hours	Novo Nordisk A/S.	Not to be mixed with other insulins Hypersensitivity
3.	Regular Insulin	Humulin R®	30-60 minutes	5-8 hours	Eli Lilly and Company	Store in refrigerator when closed and outside refrigerator when open
4.	NPH Insulin	Novolin N®	1-2 hours	14-24 hours	Novo Nordisk Medical.	May cause Hypoglycaemia, Peripheral Oedema
5.	Insulin Detemir	Levemir®	1 hour	Up to 24 hours	Novo Nordisk A/S.	Cause injection site allergic reactions
6.	Insulin U-100	Lantus®	3-4 hours	Up to 24 hours	Sanofi.	Injection needles are not reusable
7.	Insulin Degludec U-100/U-200	Tresiba®	1 hour	Up to 42 hours	Novo Nordisk A/S.	Cutaneous amyloidosis at injection May cause upper respiratory tract infection

Recombinant DNA is the technology that made gene transfer from one organism to another possible, it is a technology which was developed in order to be able to administer a human gene into the genetic material of a common bacterium, by removing a loop of its DNA known as a plasmid by a restriction enzyme and replacing it by the human gene, thus this bacterium will produce the human insulin which is harvested and administered in patients, a safer and more cost-effective than the animal insulin.[24]

The first clinically available human insulin analog known as “Lispro”, was developed and marketed by Eli Lilly and Company in 1996, it is a rapid acting analog administered through subcutaneous administration, it was developed by rearranging amino acids positions at chain B of the insulin, specifically reversal of position 28 and 29 of the amino acids,

this rearrangement reduced self-aggregation capacity of the insulin at the subcutaneous tissues, causing rapid absorption in comparison to regular insulin but both have similar activity profile and potency.[25] Several recombinant insulin preparations followed since the development of "Lispro", multiple modifications were made on the insulin structure resulting numerous compounds, as shown on the table below, depending on the needs and requirements of patients to provide convenient control of insulin levels, as some patients require emergency intervention while others need prolonged control over insulin and glucose level.

The relation between pain management and Insulin regulation is that since the nerve damage is the most accepted theory for causing and sensation of pain, then if insulin was regulated properly, it will stop or prevent accumulation of glucose, thus there will be no damage to the nerves caused by glucose accumulation, in simple words, so by preventing hyperglycaemia status in the patient, diabetic neuropathic pain can be prevented.

Thus, this method prevent neuropathic pain and not treat it after occurring, but it may help in avoiding it as well as avoiding the condition becoming worst after already occurring, by inhibiting one of the main causes of this pain, but still as some patients do not develop pain and others do without having any injured nerves, this technique is not the ultimate solution or answer to diabetic neuropathic pain or even diabetic neuropathy itself.

3.2.2 Transferring Vectors

Viral and non-viral vectors are used to deliver genetic material or therapeutic substances into the target area, different vectors have different uses and different affinities.

1. Non-viral vectors, exposed DNA particles which have more safety in comparison to viral vectors but with lower efficacy including plasmid DNA vector, this vector can be delivered without medication or associated with targeting molecule in order to improve delivery. Recently, Helixmith a South Korean biotechnology company announced results of a 12-month long study on the efficacy and safety of donaperminogene seltoplasmid injection, known as VM202 for diabetic peripheral neuropathic pain cases that took place in the US, the results showed that this injection achieved the desired end points within the acceptable safety and efficacy profiles, also showed that injection of VM202 have the potential to prevent the loss of perfusion and within 3 days after the injection it disappeared from the systemic circulation while the human growth factor gene was found even after 2 weeks of injection suggesting neuro-regeneration potency.[26]

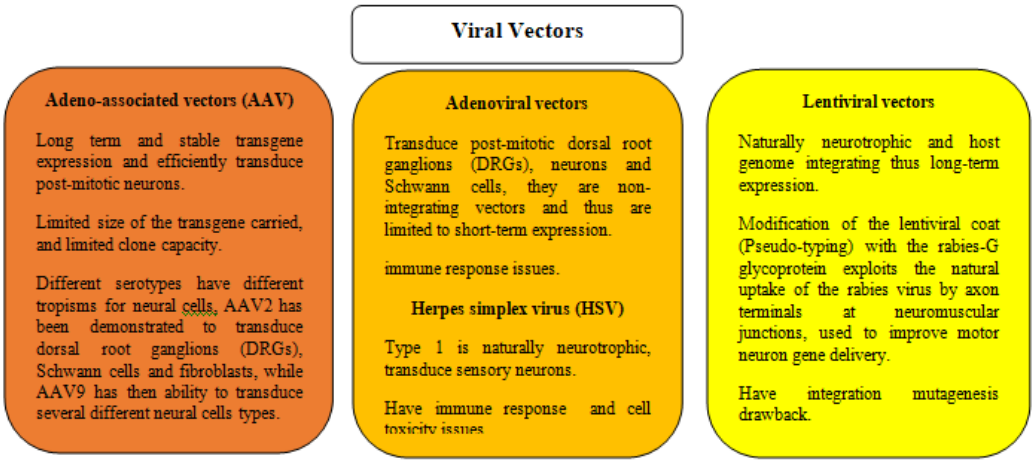


Figure (4): Viral Vectors Used in Gene Therapy.

2. Viral Vectors, small non-replicating, non-enveloped, genome-modified viruses that are administered in humans causing, they can be reengineered to deliver DNA and genetic material to target cells, they have been of great significance in different clinical trials and gene based therapeutics[27].

The viral vectors mentioned previously act mainly by delivering genetic materials required to repair damages done to different part of the nervous system, they are usually transported retrogradely through intramuscular and intraneural injections into sites with lesser invasion of target area and at an appropriate distance thus infecting both sensory and motor neurons even by vectors that are not neurotrophic in nature. By administering these vectors carrying repairing genetic materials to different parts of the nervous system, it is possible to repair damages of different nerves especially in the case of peripheral nerves, rebuild myelination of axons and restore balance in the action potential throughout the neurons and

nerve endings thus restoring the electrical activity and signalling of neurons balance which is interpreted by the control centres as pain providing relief of pain initially and preventing pain from reoccurring as the damages to nerves will be repaired.

3.2.3 Nanotechnology and Nanoparticles

Nanotechnology is a rising technique in different branches of science, recently it has been utilized in diagnosis and treatment of different health conditions. In relation to diabetic neuropathy is it also involved in the same, as blood vessels changes are related to neuropathic pain in diabetes patients, nanotechnology has been used to diagnose blood vessels damages and disease conditions using magnetic resonance imaging (MRI), and in artificially growing the blood vessels using bioprinting technology using mixtures of cells and growth factors in a suitable nano container, 3D models are achieved and thus regeneration of blood vessels could become possible as it is not possible to be regenerated by the human body by itself, this can help in prevent the loss or damage in nervous cells due to lack of blood or nutrient flow or inadequate flow.

In case of the blood vessels, uncontrolled blood glucose level lead to the formation of fat based plaques, also known as atherosclerotic plaques which affects the blood flow, nutrients and oxygen transport to the nerve cells and other organs, blood flow disruptions to the nerves lead to different conditions damaging to the nerves and nerve tissues, most commonly nerve ischemia and decrease peripheral perfusion and breakage of the basal lamina, all these factors lead to the loss and dying of nerves which is then causing imbalanced in signalling and electric pathways which is sensed by the patients as pain or discomfort.[28]

In a related application also, nanotechnology is under research now to evaluate the potential use of hydrophobic nanocoating for stents used to maintain the blood vessels' path open, preventing it from closure due to atherosclerotic plaques, thus preventing the stoppage of flow of blood and nutrients, this is especially useful as diabetic patients usually have lower levels of high-density lipoprotein (HDL) commonly referred to as the "good cholesterol" in comparison with healthy individuals so it is very possible for them to develop these plaques as this (HDL) removes and transports the low-density lipoprotein (LDL) commonly referred to as the "bad cholesterol" into the liver for reprocessing, depending on this the effect of low level of (HDL) becomes clear an at the same time the importance of stents in preventing the closure of the vessels due to low (HDL) and high (LDL), nanotechnology importance here is providing the hydrophobic nanocoating which repels all polar molecules thus preventing the formation of sludges of bacteria, glycoproteins and similar polar molecules and reduces the body's rejection of the stent which is usually identified as a foreign body by the immune system, while the other benefit of nanotechnology in this field is development of (HDL) mimicking nanoparticles such as gold particles, imitating (HDL) activity and preventing blood flow disruptions, avoiding nerve ischemia and nerve loss thus preventing pain and discomfort sensation caused by these conditions.[29]

In addition to its potential role in blood vessels, bioprinting of pre-prepared nano-scaffolds have been also under research to evaluate it nerve regeneration capabilities, in this case it is found that these nano-scaffolds have the ability to initiate an artificial pathway for a neuron's axon to grow and connect with targets beneath the damaged site by utilizing nano-fibres present alongside with growth factors present in the nano-scaffolds which can be aligned in such a way that allows the growth to occur quickly and towards the required direction, however the use of nano-scaffolds in nerve regeneration is widely related to its use in the blood vessel restoration as well, as the most common cause of nerve degeneration is blood improper flow to the nerve caused by blood vessel blockage or damage[30].

In research, few approaches have been developed to utilize nano techniques to manage this diabetic neuropathic pain related to blood vessels conditions, one is the use of chitosan based nanoparticles, obtained from alkaline N-acetylation of chitin, having the ability to form minor yet stable complexes with DNA particles (20-500 nm), upon injection of this complex in fasting rats it showed significant decrease in blood glucose level thus preventing blood vessels obstructions from occurring and allowing proper blood, nutrients and oxygen flow to the nerve and nervous tissue, while the other approach is growing blood vessels artificially utilizing bioprinting of pre-prepared nano-scaffolds in which a mixture of polymers and cells is solidified according to the shape of nano-structure used as container for growing them, this technique allows regeneration of surgical replacement of damaged blood vessels if required to restore the blood flow within the blood vessels and thus restore the balance in requirements providing for the nerves and nervous tissues.[31]

In direct relation to pain management, it was found that curcumin when encapsulated by nanoparticles had an inhibitory effect on diabetic neuropathic pain mediated by up regulation of purinergic 2 receptor (P2Y12) expressed on satellite glial cells (SGCs) which gets activated after nerve injury releasing pro-inflammatory cytokines such as interleukin-1b (IL-1b)

and connexin43 (Cx43) which increase neuronal excitability and sensitivity to pain, curcumin encapsulated by nanoparticle acts by down regulation of the (P2Y12) receptor and reduce the release of the (Cx43) and (IL-1b), these actions were found to have relieving effects on mechanical and thermal hyperalgesia on type 2 diabetic rat models.[32] From the previously mentioned examples it is clear how nanotechnology and nanoparticles have been utilized for both direct therapeutics and in delivering the therapeutics and the genetic material into the targets for their ability to enhance the bioavailability and flexibility in designing the vectors utilized to transport the required materials into the targets. Although nanotechnology and nanoparticles have great potential in treatment of many diseases including diabetes it is not challenge free, in fact there are major limitations when it comes to the use of nanoparticles, the first is that these nanoparticles are not fully understood yet, especially when it comes to their adverse and side effects, as nanoparticles have different physiochemical properties than their bulk form then the side effects will probably be different too, moreover, the action of these nanoparticles differs in the body from the action of the same material in bulk form and accordingly have different pharmacological effects on the biological system.[33]

Also under the limitations of nanoparticles, the size of these particles can allow them to cross barriers within the body that they are not meant to supposed to, which will create issues and undesirable adverse effects that are very difficult or counter especially in the case of blood brain barrier for example, whereas these particles can reach the brain's pleasure centre as they can result in severe habit forming effect even with drugs that are not recreational in nature or the nanoparticles could be of a material that can be toxic to the brain cells and tissues. Finally, these mentioned limitations alongside with their high cost, lack of knowledge about their excretion from the patient's body, drug-drug/food interactions compatibility, and the fact that is according to the American food and drug administration (FDA) 92% of potential drugs pass pre-clinical experiments but fail in clinical trials which strongly suggests that nanoparticles-based medicines work differently in humans than they do in animals and in vitro experiments, meaning that it is not possible to accurately predict their behaviour, exact action and side effects.

3.2.4 Mesenchymal Stem and Stromal Cells

Mesenchymal stem and stromal cells neuroprotective or neuro-regenerative properties to counter different types of damages to the nerves caused by diabetic complications, stem cells have the ability to differentiate into various types of cells, which means it have the potential to replace damaged nerves that causes pain and discomfort for diabetic patients, in recent studies it was found that intramuscular administration of mesenchymal stem cells was able to conduction velocity, blood flow and the density of small vessels of sciatic nerve conduction in the muscles of streptozotocin (STZ)-induced diabetic rat models, all of these action are related to providing a relief from pain and discomfort feeling in that specific nerve and the organs it controls, alongside with immunosuppressing and anti-inflammatory effects of these cells on the peripheral nerves in the same models.[34]

Other suggestions are that the benefits of the mesenchymal stem and stromal cells are obtained from locally released angiogenic factors, such as vascular endothelial growth factor and basic fibroblast growth factor, as these cells secrete neurotrophic and angiogenic factors aiding in the regeneration processes of the damaged pain causing nerves.

Bone marrow mesenchymal stem cells or stroma cells on the other hand has been a topic of interest, it was found in a study a while ago that these cells contain high amount of galanin peptide with it receptors, the galanin receptor 2 (GalR2) is the most present among the receptors, administration of these cells intravenously in animals was found to help increasing the expression of growth factors, galanin is known of having a significant role in development, survival and growth of peripheral and central neurons[35]. Adipose stem cells administered intravenously in an induced neuropathy rat model with increased levels of plasma's vascular endothelial growth factor (pan VEGF-A) with up-regulation of its isoform (VEGF165b) is in the spinal cord was found to be reduced by the effect of these adipose stem cells, also reduced by intraperitoneal administration of (bevacizumab) an anti-VEGF-A monoclonal antibody, thus adipose stem cells are used to treat pain originating from spinal cord changes as well as vascular changes as per the growth factor, this could be a novel approach in providing relief of pain after the necessary research and testing for safety and efficacy is completed.[36]

3.2.5 Miscellaneous Agents in Gene Therapy of Painful Diabetic Neuropathy

There are a few agents that are utilized in management of pain targeting causes of pain at genetic bases, although they are of different classes and categories, and do act by different modes, they can all be used or have the potential to be used as pain relieving agents.

1. Anti-Inflammatory Agents, for example interleukin-10 (IL-10) is a non-viral cytokine that possessing neuroprotective effects towards inflammation and physical injury in the central nervous system, it has the potential to decrease expression of pro-inflammatory cytokines along with decrease of antigen presentation, and promoting neuroprotection[37], and the Interleukin-35 (IL-35) which was recently found to have the ability to promote microglial polarization of the (M2) anti-inflammatory phenotype over the (M1) pro-inflammatory phenotype at the highly aggressively proliferating immortalized cells (HAPI) through inhibition of c-Jun N-terminal kinase (JNK) signalling pathway and activation of JNK/signal transducer and activator of transcription 6 (STAT6) human gene pathway.[38]

2. Soluble Epoxide Hydrolase (sEH) Inhibitors, it is a cytosolic enzyme targeting degradation of epoxy-fatty acids into dihydroxy-fatty acids and plays an important role in inflammation, for example 1,3-disubstituted urea is the most preferred (sEH) inhibitors for its high affinity, these inhibitors stop the degradation of epoxy-fatty acids and thus prevent the inflammation status in chronic inflammation cases, especially in those related to the nervous tissue where the inflammation can be very painful.[39]

3. Potassium/Chlorine (K+/Cl-) cotransporter (KCC), at the spinal cord level, neuropathic pain can develop because of loss of synaptic inhibition because of gamma aminobutyric acid (GABA) and dysregulation of chloride due to nerve injury, (KCC) gene delivery to the spinal cord initiate restoration of chloride thus restoring the normal synaptic inhibition providing a long term relief of the pain caused by this mechanism.[40]

4. Coumarin Derivative (Osthol), This derivative was found to be able to down regulate purinoceptor 4 (P2X4) receptor on satellite glial cells (SGCs) and deactivate these cells in the dorsal root ganglia preventing activation and release of different pro-inflammatory mediators.[41]

5. Tanshinone IIA, a traditional Chinese herbal medicine component that has been under study for having potential to counter inflammation and neuropathic pain by inhibiting high mobility group Box (HMGB1) protein, and activating erythroid-derived 2-like 2 (Nrf2), having a number of antioxidant elements opposing oxidative stress and preventing it from damaging nerves and causing polyneuropathic pain across the patient's body.[42]

6. Quercetin, this natural occurring plant pigment have shown analgesic effects in reducing pain sensations in genetically mutated and excessively obese mice of (db/db) model, quercetin full mechanism of action as an analgesic is not fully understood yet but in a recent experiment it was found that it is mainly acting by changes of synaptic morphology and protein level in the dorsal horns of the spinal cord of the (db/db) mice through inhibition of by inhibiting rapamycin/ribosomal protein S6 kinase (mTOR/p70S6K) signalling pathway. [43]

7. Neuronal Nitric Oxide Synthase (nNOS) Inhibitor, administering of 7-nitroindazole (7-NI) in experiments on rat models was able to alter nociceptive transmission reducing the pain resulting from nerve injuries.[44]

TABLE III: Summarize of Gene Therapy Based Treatments for Diabetic Neuropathic Pain

S.No	Method Name & Type	Mechanism of Action	Recent Status/Advancement	Remarks
1.	Recombinant DNA Insulin Analogs (Indirectly involved in pain management).	Maintain and regulate blood glucose level in the body, preventing it from causing a state of hyperglycaemia which damages the nerves resulting in sensation of pain and discomfort.	Products available and marketed, example, Lispro (Humalog®) from Eli Lilly and Company.	This method avoids the causes of pain sensation but does not help in treating of providing relief of occurring pain.
2.	Non-viral transferring Vectors (Directly involved in pain management).	Transfer genetic material into the targeted cells using non-organic carriers for repairing or replacing. Recent candidate VM202 showed potential to prevent the loss of perfusion and human growth factor gene presence after injection up to 2 weeks suggesting neuro-regeneration ability.	Donaperminogene seltoplasmid injection (VM202) by Helixmith Co., Ltd. Successfully passed phase 3 extension study (DPN 3-1b) October 2019.	This method has the potential to regenerate damaged nerves, but the pain-relieving effect shows after minimum 6 moths of injection, no immediate relief.
3.	Viral Transferring Vectors	AAV and HSV are the most used among the viral vectors.	KLS-2031, an adeno-associated virus containing	Long effect of analgesia with lesser

	(Directly involved in pain management).	Transfer genetic material into the target cells using organic carriers. Transgene expression with potential for effective transfer to the nervous system. HSV gene delivery has potential to enhance the delivery of genes the sensor ganglions.	glutamate decarboxylase 65, (GAD65) glial cell-derived neurotrophic factor (GDNF), and interleukin-10 (IL-10) approved by U.S FDA for human clinical trials February 2020. Opioid receptor up-regulation and effective transducing in herpes simplex virus delivery.	dose required is expected from KLS-2031; however, it is in the initial phase of clinical trials, side effects, safety, efficacy in humans to be determined. cell toxicity and immune response issues.
4.	Nanotechnology and Nanoparticles (Both directly and indirectly involved in pain management).	Nano-scaffolds for blood vessels artificial growth to replace damaged ones. Nanocoating for stents administered surgically to maintain open pathways in blood vessels. Gold nanoparticles mimicking the (HDL) action to remove (LDL). Chitosan nanoparticles forming complexes with DNA reducing blood glucose level. Curcumin nanoparticles down-regulate purinergic 2 receptor (P2Y12).	For Nano-scaffolds research is going on regarding the further use of gelatine-vinyl acetate and poly-ε-caprolactone for blood vessels engineering. Nanoparticles are more used now in research for potentiality in downregulate purinergic 2 receptor family, including some particles derived from traditional Chinese medicine system.	As it is a new technique, side and adverse effects needs to be further studied to decide safety and efficacy profiles.
5.	Stem Cells (Indirectly involved in pain management).	Replacing therapy for damaged nerves which cause pain and discomfort. galanin peptide and receptors in bone marrow stem cells and growth factors in adipose stem cells. Regeneration of nerve cells prevent pain from occurring.	Bone marrow and adipose-derived stem cells under investigation for regenerative and protective capacities.	Stem cells is applied in therapeutics usually, but regeneration potential for nervous system is the main interest in today's research.
6.	Anti-Inflammatory Agents (Directly involved in pain management).	Counter inflammatory mediators and prevent inflammation and associated pain.	Interleukin-35 (IL-35) recent discovered ability to change microglial polarization of the (M2) over (M1) phenotype.	(M2) is anti-inflammatory. This class is related to central nervous system inflammation.
7.	Soluble Epoxide Hydrolase (sEH) Inhibitors (Directly involved in pain management).	Epoxy-fatty acids into dihydroxy-fatty acids convert inhibitors, thus it prevents inflammation and pain.	High affinity 1,3-disubstituted urea is the most preferred (sEH) inhibitors .	Effective but slow pain relief treatment.
8.	Potassium/Chlorine (K+/Cl-) cotransporter (KCC) (Directly involved in pain management).	Gene delivery to the spinal cord. Restoration of chloride and restoring the normal synaptic inhibition providing a long-term relief of the pain.	Preclinical trials still going on.	Spinal cord injury treatment specific.
9.	Neuronal Nitric Oxide Synthase (nNOS) Inhibitor. (Directly involved in pain management).	Nociceptive transmission preventing pain and down-regulate neuronal nitric oxide synthase.	Preclinical trials still going on.	Decrease hyperalgesia within 1 to 2 weeks, needs more data from the preclinical trials.

10.	Bioflavonoid (Both directly and indirectly involved in pain management).	Quercetin naturally present in plants was able to promote changes of synaptic morphology and protein level in the dorsal horns of the spinal cord, by inhibiting of (mTOR/p70S6K) signalling pathway.	Promising results in during animal testing.	A method to prevent and treatment of neuropathic pain
11.	Aldose Reductase Inhibitors (Indirectly involved in pain management).	They inhibit polyol pathway preventing reduction of glucose into sorbitol which initiate osmotic pressure capable of damaging nerves	Many candidates such as Lrestatin withdrawn from clinical trials due adverse effects or no useful effect.	Novel drugs with higher efficacy and safety is required

4. CONCLUSION

Diabetic neuropathy and neuropathic pain are serious complication of diabetes mellitus, these conditions have been deemed untreatable by conventional therapeutics or were able to provide only a minor or short term relief of pain which was not able to completely inhibit the pain sensation for patients, thus there was a need to develop more advanced techniques of therapy which have the ability to provide longer term and more significant pain reduction for diabetic patients.The future of this treatment does have high potential but with a lot of challenges also, scientists are now researching and experimenting on different candidate agents that can provide the required therapeutic effects without or with minimum adverse effects and with better safety and efficacy profiles alongside with faster pain managing action as well as more cost effective treatments, it is hoped that at some point they can develop an agent capable of provide prolonged relief of pain as a start before developing an agent capable to treat the cause of pain by a much shorter time requirement than the current agents have, all of this however is still under initial research. For neuropathic pain in diabetic patients, although not the case in all the conditions, but the majority of painful cases caused by damaged nerves, these are usually unregenerable making the condition permanent and continuously being worst, but with recent techniques showing regenerating capabilities, it might be the first steps taken towards normalizing the treatment and reversing of nerve injuries providing a solution not only for diabetic neuropathic pain but for all other diseases and conditions that are caused by nerve damage. This field now have global interest and potential, thus there is a need for funding and performing multiple research experiments and trials to understand it mechanism better and evaluate its possibility to be used in the future, however, this will require large funding and authorizations from different agencies to utilize animals first as tests subjects in preclinical evaluation and only after successfully clearing those trials it can proceed for human clinical trials.

Acknowledgement

Authors are thankful to Lovely Professional University for providing suitable environment to complete this manuscript.

Conflict of interests

None

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