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A Comprehensive Review on Significance of Biomarkers in the Treatment of Diabetic Peripheral Neuropathic Pain

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Abstract

The most prevalent kind of neuropathy in people with both type 1 and type 2 diabetes is diabetic peripheral neuropathy (DPN) affecting over 90% of the diabetic patients, contributing to substantial morbidity and mortality. Clinically, this neuropathy might cause symptoms from motor, sensory, or autonomic nerve dysfunction, or it can be asymptomatic. Every diabetic neuropathy is asymptomatic, increasing the possibility of foot ulcers and amputation. Lower limb amputation is more common in cases with these ulcerations. Kinesthesia, tingling lower leg, burning sensation in feet altered patient sleep and mood particularly at night, are characteristics of DPN. The discovery of novel treatment targets may result from biomarker research by deepening our understanding of the molecular underpinnings of a disease process. In this review, biomarkers associated with DPN have been identified. A review of the biomarkers associated with diabetic peripheral neuropathy and their potential as treatment targets.

Keywords: Diabetic peripheral neuropathy, Biomarkers, Kinesthesia, Neuropathic pain.

1. INTRODUCTION

Diabetic Peripheral neuropathy (DPN) is a microvascular complication of diabetes which accounts for significant morbidity and mortality. Symptoms includes sensory defects and Peripheral neuropathy affects 30 to 90% of diabetic people [1]. All diabetic neuropathies are asymptomatic which can result in risk of foot ulceration and amputation. Foot ulceration brought on by trauma that is invisible to the patient may be the final result of such sensory impairments affecting the lower limbs. In fact, it's been reported that foot issues account for 20% of diabetes hospital admissions in the United States. According to Parkhouse and Le Quesne (1988)[2], these ulcerations increase the risk of lower extremity amputation, and the rate of lower limb amputation in diabetics is fifteen times greater than in non-diabetics.

Diabetic neuropathy (DN) may be defined as a type of nerve damage in people suffering with diabetes mellitus (DM), has different phenotypic, clinical trajectories, and anatomic aspects. Hyperglycemia and microangiopathy result in a shared underlying etiology. Even now, DN is

associated with morbidity and is believed to raise the risk of developing diabetic foot syndrome and falling because of impaired balance, particularly in individuals over 60[3]. Additionally, there is a substantial risk of infection and amputation with diabetic foot syndrome[3]. Management of metabolic factors and glycaemic control is essential for either avoiding or postponing DPN.

While there is no cure for DPN once it has started, there are medications that may be applied topically, such as topical medications, antidepressants, anticonvulsants, and opioids, that can help manage the symptoms. However, patients frequently receive prescriptions for fewer drugs than what is advised, which results in inadequate management of DPN symptoms and treatment cessation. The pathophysiological mechanisms of the biomarkers that are presently thought to cause DPN are covered in this review, along with preventative measures and therapy options for DPN symptoms.

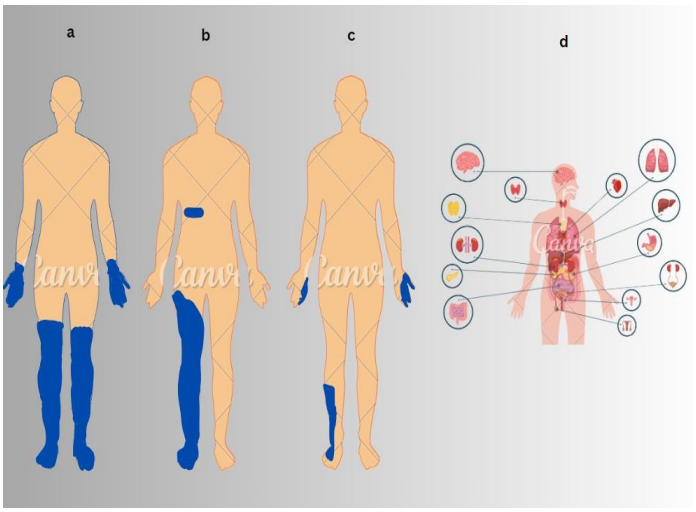

Figure 1 - Different patterns of neuropathy in Diabetic patients. a: small-fibre-predominant neuropathy; b: radiculopathy; c: mononeuropathy; d: autonomic neuropathy.

2. PATHOPHYSIOLOGY

The most prevalent etiological cause for peripheral neuropathy (PN) is diabetes, which has also been the subject of the greatest pathogenesis research. A recent study found that individuals with reduced glucose tolerance may experience PN, a prediabetic condition, despite the prevalent idea that chronic hyperglycemia causes the problems associated with diabetes, including neuropathy. In comparison to age-matched controls from the general population, The study found that poor glucose tolerance was twice as common in a group of persons with chronic idiopathic polyneuropathy[4].The pathophysiology of DPN includes oxidative stress (Superoxide dismutase, lipid peroxidation), accumulation of advanced glycation end products (AGEs), polyol buildup, decreased nitric oxide/disrupt endothelial function, impairment of Na+/K+-ATPase activity, and homocysteinemia.

Table 1. Diabetic neuropathy's pathophysiological components

Increase sorbitol and Fructose.
Increase in oxidative stress (superoxide dismutase, lipid peroxidation) as well as Advanced glycosylated end products(methylglyoxal)
Decrease Na+/K+-ATPase activity.
Decrease in the amount of free carnitine and myo-inositol.
Increase protein kinase C activation.

Decrease nitric oxide and increase homocysteine: Impaired endothelial function.
Increase reactive oxygen species.
Increase NADH/NAD+

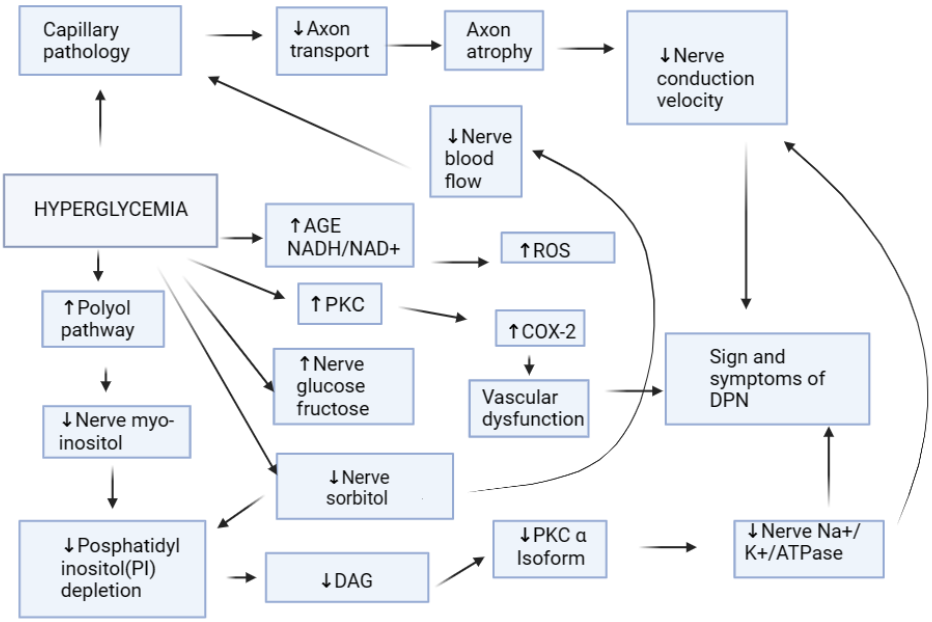

Figure 2: Pathophysiology involved in diabetic peripheral neuropathy

3. MECHANISMS OF DIABETIC NEUROPATHY DEVELOPMENT

Pathogenic pathways linked to diabetic neuropathy include metabolic abnormalities, microvascular damage, changes in neural and immune system linkages, and activation of glial cells. The pathways polyol, advanced glycation end products, inflammation, hexosamine, protein kinase C, poly (ADP-ribose) polymerase, and oxidative stress are among the mechanisms. An illustration of one such system is depicted in Figure 3.

3.1 POLYOL PATHWAY

The body breaks down excess glucose via the polyol or sorbitol pathway. Aldose reductase, an enzyme that is a rate-limiting step in this pathway, glucose gets converted to sorbitol, which is subsequently oxidized by the enzyme sorbitol dehydrogenase to fructose, a strong glycating agent. The excessive increase in sorbitol inside of cells reduces the amount of taurine and myoinositol in the nerves and disrupts the activity of the Na + / k + -ATPase membrane, which results in an accumulation of Na+ in the nerve, axon impairment, and structural damage to the nerves [56]. Free amino acid which is present in proteins, lipids, and nucleic acids gets glycosylated in the membrane of endothelial cells which altermolecular structure and function[7] which leads to diminish vasodilation. Additionally, the building up of AGEs bind to the AGE receptor which is present on macrophages, causing the production of adhesion molecules like VCAM-1, tumor necrosis factor, and inflammatory cytokines like IL-1[8]. Nuclear enzyme poly (ADP ribose) polymerase (PARP) activation is another mechanism that leads to diabetes problems. DNA damage and PARP 1 activation are brought on by high levels of oxidative stress, and both processes are crucial to the pathophysiology of DPN [9].

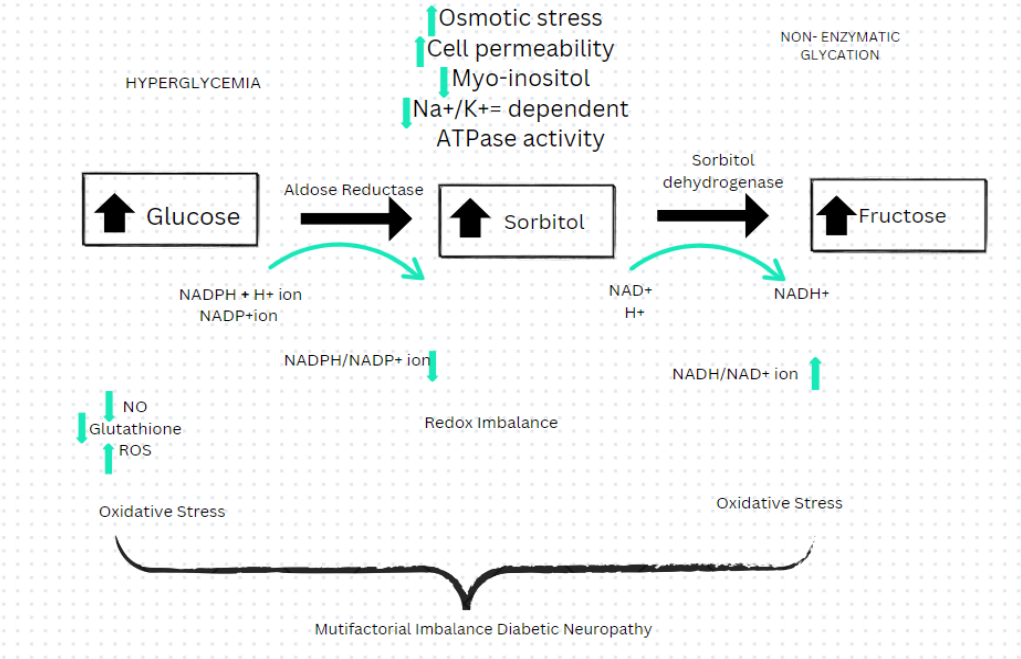

Figure 3 - Polyol pathway

4. BIOMARKERS INVOLVED AND AS POTENTIAL THERAPEUTIC TARGET

An objectively measured and assessed trait that serves as a marker for pathogenic processes, normal biological processes, or pharmacological reactions to a therapeutic intervention is what is known as a biomarker[10].

4.1Enzyme as biomarkers

4.1.1 Aldose reductase

Aldose reductase, it is a member of the superfamily of aldo-keto reductases., is a cytosolic enzyme that is reliant on monomeric reduced NAD phosphate (NADPH). (EC number 1.1.1.21)[11]. The enzyme that sets a rate limit is aldose reductase. It uses NADPH as a cofactor in the polyol pathway to convert glucose to sorbitol[12]. A higher sorbitol concentration through the polyol pathway accumulates and raises osmotic stress, which in turn affects the Schwann cells[13].Fructose and sorbitol, byproducts of the polyol system, build up and impair nerve conduction velocity, intracellular sodium deposition, axon swelling, and the Na+/K+ ATP pump[14].

Aldose reductase increases the activity of MAPK in the spinal cord, nerves, and dorsal ganglia[13]. Poly-ADP ribose polymerase (PARP) and protein kinase activate p53 and NF-kB activator protein-1 to regulate gene expression, which in turn upregulates several inflammatory genes, COX-2 endothelin-1 activity, and inducible nitric oxide synthase (iNOS). An extended polyol pathway, which is aided by aldose reductase, better glycation by-products, improved oxidative stress, and changed properties of protein kinase C, results in neuropathy. In addition to the polyol pathway, another mechanism that contributes to the genesis of diabetic neuropathy is enhanced flux via the hexosamine route[15],[16].

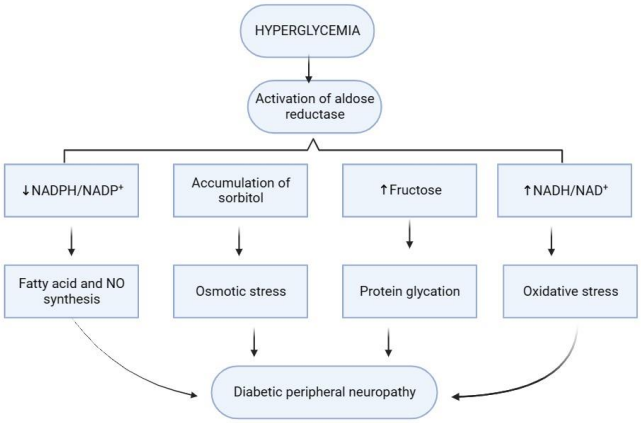

Figure 4 - Role of Aldose reductase in DPN

4.1.2 NOX

The enzyme nicotinamide adenine dinucleotide phosphate oxidase (NOX) is the reason for the generation of reactive oxygen species during hyperglycemia. RAC1 and other cytosolic NOX components are phosphorylated, such as p47phox and p67phox, is the primary mechanism that converts oxygen into reactive oxygen species during hyperglycemia. Overexpression of NOX not only activates the hexosamine pathway, protein kinase C, AR, and activation of AGE pathway, but it also promotes hyperlipidemia, collagen synthesis, and the stimulation of inflammatory cytokines including TGF-β, TNF-α, NF-kβ, IL-6, and IL-18, as well as endothelial growth factors like VEGF and FGF. Therefore, the emergence of problems related to diabetes is associated with overexpression of NOX. AGE, poly (ADP-ribose) polymerase, polyol, and protein kinase C (PKC) are among the mechanisms.

Hosseini and Abdollahi (2013) [17] claim that these pathways activate NOX, therefore causes the formation of inflammatory indicators including oxidative stress enzyme-like PARP, NF-kβ, p38 MAPK, cyclooxygenase-2 (COX-2), and 12/15-lipoxygenase. Neuroinflammation and neuronal degeneration are the outcomes of this. The NOX enzyme causes reactive oxygen species to be produced during hyperglycemia and contributes to the development of DN[18]. Drug such as metformin, rapamycin may act as NADPH-oxidase inhibitor[19 20].

4.2 Epigenetic biomarkers

4.2.1 Micro RNA

A class of evolutionary conserved regulatory non-coding RNAs known as microRNAs (MiRNAs) possess a length of around 20 nucleotides, less than 200 nucleotides, and they are complementary to different messenger RNAs' (30-UTRs). Targeted mRNA binding to miRNA causes mRNA cleavage and stops translation[21] [20]. In models of neuropathic pain in animals, dysregulation of a large number of miRNAs occurs. It has been revealed that miRNAs—miR-9, miR-92, miR-93, miR-155, miR-141, miR-146a miR-30b, miR-155, miR-132-3p and miR-106a—are strongly linked to PDN.

Table 2: miRNAs related in Diabetic peripheral Neuropathy

S.NO	MiRNAs	Target	Functions	References
1.	Mir-9	CALHM1	increases Ca2 + influx and ATP generation in PDN rat neurons, which activates the ATP-P2X7R signaling pathway.	2223
2.	Mir-92	KCC2	Pain is muted by upregulation of KCC2 at the spinal cord, which balances the concentration of Ca2 +. In (PDN), downregulation of KCC2 leads to a reversal in chloride neuronal flow, which in turn causes a lack of GABA-mediated pain inhibition.	24,25,26
3.	Mir-30b	Nav 1.7	Regulates the pain sensitivity	27,28,29
4.	Mir-132-3p	Glu A1 & Glu A2	pro-nociceptive impact in neuropathic pain that is persistent	30,31
5.	Mir-106a	12/15 Lox	controls oxidative stress, nitrative stress, and cell apoptosis in PDN	32
6.	Mir-93	STAT3	involved in controlling the expression of inflammatory cytokines and the development of neuropathic pain	33,34,35
7.	Mir-155	SOCS1	MAPK activation, Inflammation	36,37
9.	Mir-141	HMGB1	Increased miRNA expression reduces neuropathic pain	38,39
10.	Mir-146a	NF-Kb	miR-146a increases the expression of TNF-α and IL-1β via decreasing the inhibition of NF-κB p50 and p65	40

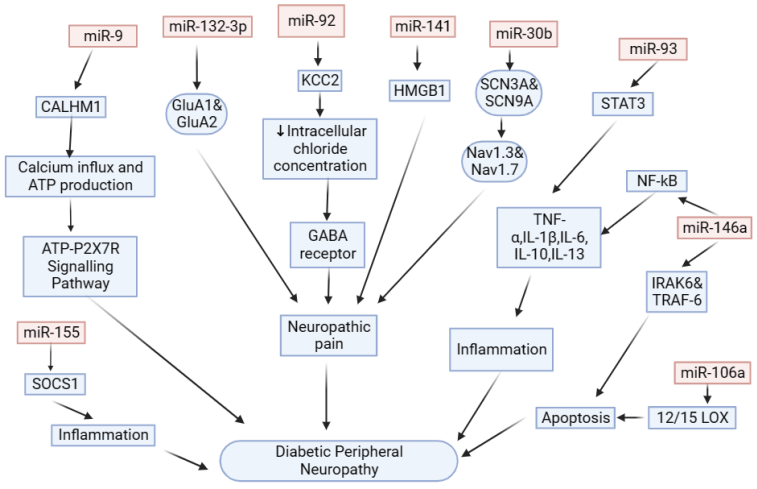

Figure 5 - miR involved in DPN

4.2.2 DNA methylation

When a methyl (CH3) group binds to DNA, DNA methylation takes place. Methyl (CH3) group attaches itself permanently and reversibly to a cytosine at palindromic locations called CpG islands. DNA methyltransferases (DNMTs) are a class of enzymes that control gene activity through the methylation process. These sites are often found in or close to promoter

and regulatory regions [41]. DNA methylation controls the expression of genes and is crucial for several biological processes, including the vulnerability to illness[42]. DNA methylation at important genomic regions linked to diabetes problems has persisted over time, according to a genome-wide methylation study of samples taken from the same diabetic patients 16–17 years apart [43]. One potential biomarker for PDN in white blood cells is significantly reduced whole genomic DNA methylation. Hyperglycemia is changing the PDN-associated gene expression patterns and DNA methylation state[44].

For example, Since the NINJ2 protein in Schwann cells induces nerve regeneration following damage, methylation of the ninjurin 2 (NINJ2) gene downregulates its expression and may contribute to the development of neuropathies[45]. Additionally, reduced methylation of genes associated with change in neuronal polarity, such as BR serine/threonine kinase 2 (BRSK2), albeit this was not confirmed. A noteworthy discovery made during the same investigation revealed a significant hypomethylation in the 50UTR region of the claudin 4 (CLDN4) gene. Despite its intended role of facilitating transcriptional factor attachment, it was linked to a notable dysfunction of gene expression. The authors proposed that reducing gene expression might be caused by additional epigenetic processes[46].

PDN progression is associated with certain DNA methylation patterns via pathways pertaining to glycerophospholipid metabolism (phospholipase A2 and phosphatidylserine decarboxylase), and MAPK signaling may be implicated, according to a genome-wide analysis of DNA methylation in the sural neurons of patients with variations in PDN formation[47]. Moreover, DN potential genes and pathways (PI3K-Akt signaling, ECM regulation, and immune response) were found by means of a combined study of the sural nerve transcriptome and methylome[48]. Guo et al. observed numerous CpGs inside regulatory areas that are differently methylated that can affect the production of miRNA or lncRNA. The miR3138 gene, which targets erb-b2 receptor tyrosine kinase 4 (ERBB4), has the highest methylation difference and has been linked to the advancement of PDN[49].

4.2.3 Changes to histone after translation:

The dynamic processes of DNA wrapping in nucleosome production and chromatin architecture are governed by post-translational histone changes., modifications such as acetylation, methylation, and others. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) are the two categories of enzymes that control the most common mechanism, histone acetylation[50]. HDAC inhibitors disrupt the acetylation process epigenetically, contributing to neuropathic pain [51]ropathic hypersensitivity brought on by peripheral nerve damage[52], as well as diabetic retinopathy and nephropathy[53],[54]. HDACs are targets for the treatment involved in DPN. Research on nerves in the peripheral region from diabetic animal models and Schwann cells (RSC96) grown at high glucose levels in vitro revealed that HDAC1 and STAT3 are viable targets for preventing PDN by increasing autophagy[55].

4.3 Inflammatory biomarkers

4.3.1 COX-II

The pathophysiology of DPN has also been linked to glucose-mediated activation of the cyclooxygenase (COX)-2 pathway. Prostaglandin (PG) synthesis and function are compromised by COX-2 overexpression, which also triggers downstream inflammatory responses. It has been previously documented that experimental diabetes causes an upregulation of COX-2 in peripheral nerves and dorsal root ganglia (DRG) neurons. Additionally, targeted COX-2 inhibition and/or COX-2 gene deletion offer protection against

a range of DPN impairments[56,57]. The pathophysiology of experimental diabetic neuropathy, it has been previously shown a connection between hyperglycemia and COX-2 activation[57][75]. Numerous studies have demonstrated that one important mechanism underlying peripheral nerve damage in diabetes is the activation of the COX-2 pathway. For instance, we've found that diabetic rats' peripheral nerves show higher levels of COX-2[57].

Additionally, research have indicated a clear correlation between overexpression of COX-2 and activation of aldose reductase (AR) or PKC caused by hyperglycemia, two important pathways leading to peripheral nerve injury[58,59]. For instance, it has been shown in several studies that, in models of human colon cancer and experimental diabetes, increased AR flow stimulates downstream COX-2 activation, which AR inhibitors can prevent[60,61]. Nevertheless, other studies demonstrated that targeted COX-2 inhibition cause a significant decrease of AR expression and stopped aldose reductase-metabolites from building up in renal inner medulla cells [62].

This implies that downstream metabolite production and AR gene expression may be impacted by pharmaceutical modification of the COX-2 pathway. Additionally, prostaglandin G2 is converted to prostaglandin H2 in a peroxidase-dependent manner upon activation of the COX-2 pathway. This process promotes the generation of superoxide, which leads to lipid peroxidation and protein nitrosylation[63]. Elevated superoxide concentrations, such as NO, encourage the development of peroxynitrite, a highly reactive oxidant, which is more common in diabetes[64,65], which causes nitrosative stress. One detrimental consequence of nitrosative stress has been shown to be the production of cyclooxygenase-2, intuitive nitric oxide synthase, cell adhesion molecules, and other inflammatory mediators. Protein nitration and nitrosylation[66], mitochondrial malfunction[67], poly (ADP-ribose) polymerase[68], and the stimulation of MAPK are additional harmful downstream effects[69]. Peripheral nerve impairment and diabetic vascular disease is directly related to all of these pathways.

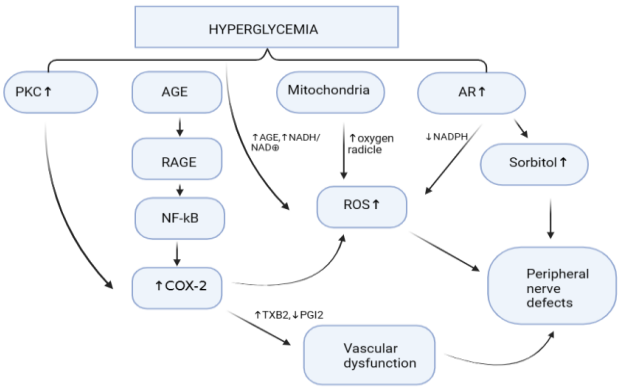

Figure 6 - Activation of secondary mediators through hyperglycemia.

This figure denotes how hyperglycemia activates COX-2 Increased auto-oxidation, aldose reductase (AR) pathway activation which cause depletion of NADPH and NAD+, activation protein kinase C (PKC) activity, activation of AGE pathway, and elevated reactive oxygen species generation in both mitochondria and nonmitochondria, hyperglycemia triggers the expression of COX-2 [70,71].

4.3.2 TNF-*α*

Tumor necrosis factor-α (TNF-α) is a monokine that was initially shown to be produced by macrophages. In vivo production of TNF-α gets elevated by chronic hyperglycemia. The mechanisms behind elevated production of TNF-α may be linked to contact with advanced

glycation end products (AGEs)[72], hyperglycemia-induced oxidative stress[73], or macrophage activation by high glucose itself[74],[72]. TNF-α production that is elevated in vivo has the potential to worsen insulin resistance[75] and ultimately lead to diabetes complications.

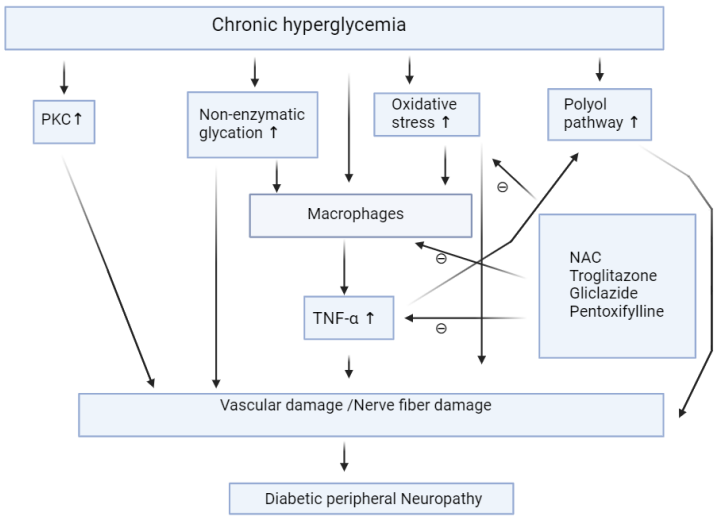

Figure 7 - Potential function of TNF-α in the development of diabetic neuropathy.

Cloliclazide: an antidiabetic drugs that reduce TNF-α production[76]. Troglitazone, regardless of blood glucose levels, improved reduced MNCV and the aberrant morphology of peripheral nerves in diabetic rats, as was the case with NAC[77]and gliclazide[76,78]. Troglitazone has been linked to increased lipid oxidation and decreased generation of TNF-α in blood and nerve tissues[78].

N-acetylcysteine (NAC), which is employed as an antioxidant in therapeutic settings to treat detoxication and is crucial to cell redox reactions[79,80]. It has been observed that the free-radical scavenger NAC inhibits the generation of TNF-α both in vivo and in vitro[81].

In diabetic rats, pentoxifylline prevented increased TNF-α production and lowered MNCV, since it was known to limit TNF-α production and its effect [82].

4.3.3 IL-6

Interleukin-6 which is released by both glia and neurons, the progenitor cytokine of the neuropoietin family, which functions as a neurocytokine in both regeneration and injury scenarios [83]. Peripheral nerve health not only depends on Schwann cells, but also on the myelin-producing cells. There are an increasing number of studies examining the effects of IL-6 which is present on Schwann cells as well as on myelin expression. Rat expression studies[84] demonastrated that myelinating Schwann cells at the Ranvier nodes and other membrane regions of the internodal cytoplasm produce IL-6 receptor alpha (IL-6Rα). The expression pattern points to a possible function for IL-6 in preserving the connection between the nerve axon below and the myelinating Schwann cell.

Furthermore, following sciatic nerve damage, IL-6Rα expression is increased, especially in the remyelination stage of the injury, indicating a function in the regeneration stage [84]. One purpose of denervated Schwann cells' early IL-6 release is to promote monocyte recruitment, most likely through LIF regulation, to remove myelin debris and axons, which is a necessary condition for effective regeneration [85].

Exogenous administration of low-dose IL-6 can treat DPN may be beneficial due to (1) heightened muscle insulin sensitivity[86], (2) reduced inflammatory response throughout the

body[87,88], (3) enhanced axon remyelination[89], (4) enhanced regeneration of nerves[90], (5) enhancement of lipolysis [91], and (6) reduced production of insulin [92,91]. In preclinical DPN models, acute exogenous delivery of low-dose IL-6, dosed to mimic exercise-induced IL-6 kinetics, has demonstrated substantial peripheral nerve protection and repair. To further explore the neuroprotective and regenerative potential of recombinant human IL-6 (Atexakin® Alfa), a low-dose, pulsatile delivery system is now being developed.

4.4 Genetic risk factors

A genetic-metabolic model developed by Witzel et al. should aid in determining the etiology of diabetic neuropathies. There is evidence linking DPN and DN to polymorphisms in ACE (angiotensin converting enzyme) an enzyme and vasoconstrictor, APOE (apolipoprotein E) a gene, MTHFR (methylene tetrahydropholate reductase) an enzyme involved in the metabolism of homocysteine and the folate cycle, NOS3 or ENOS (nitric oxide synthase 3 or endothelial nitric oxide synthase) a gene, and VEGF (vascular endothelial growth factor) a chemokine[93]. The highlighted genes are all engaged in important biochemical pathways that have been connected to diabetes and associated consequences, the authors stressed[94,95].

An essential function of the ACE, an enzyme and vasoconstrictor, is to convert angiotensin I into angiotensin II in the renin-angiotensin systemApart from its many activities such as oxidative stress, inflammation, and vascular changes, angiotensin II is also connected to the regulation of insulin and glucose levels[96,97]. Polymorphism of the gene ACE I/D in DN was shown by several independent studies and meta-analyses, demonstrating that the D allele was a cause, especially for DPN[96,98,99,100]. The polymorphism at locus ACE I/D has been suggested as a possible pharmacogenetic marker since it influences statins, ACE inhibitors, and angiotensin receptor blockers—all of which are often used in the treatment of diabetes. For this reason, the therapeutic value of regular ACE I/D genotyping remains controversial[9310196].

Neuronal regeneration and repair, as well as lipid metabolism, are impacted by the LDL receptor ligand and cholesterol transporter APOE. Among many Single nucleotide polymorphisms (SNPs), two functional SNPs such as rs429358 C/T and rs7412 T/C, known by the allele variants such as e2, e3, and e4 in the APOE gene. These SNPs result in amino acid substitutions at the protein level of 112Cys/Arg and 158Arg/Cys, respectively[93,101].

While the e4 allele exhibits the opposite effects, the e2 allele is often linked to lower levels of LDL cholesterol and greater circulatory APOE. Neuropathology risk mutation was observed in e4 allele and the APOE genotype has link with diseases of the central as well as with the peripheral nervous systems.

Research indicates certain demographic specificities regarding the involvement of APOE in DM as well as in DN. The e4 allele has been identified by meta-analyses as a causative agent for DPN, and data about extremely severe DPN in the subject with e4 carriers have also been reported [93,101,102,103].

Furthermore, pharmacogenetic assessment of APOE is a hot issue right now. Metformin, an anti-diabetic medication, has been shown to increase APOE expression, which in turn promotes neuron regeneration. According to some research, DM patients with APOE e4 type had a poorer reaction to statins but a greater response to lifestyle modifications. Although these results are currently not consistent, they may have implications for several elements of the monitoring and control of DM and DN[93].

MTHFR, which ia an enzyme involved in the metabolism of homocysteine and the folate cycle. It also plays a part in inflammation, immunological response, neurotransmitter synthesis, and

protein synthesis. MTHFR is necessary for the remethylation of homocysteine to methionine., and decreasing MTHFR activity is linked to hyperhomocysteinemia, which harms blood vessels, lipid metabolism, brain function, etc. [9396]. High homocysteine levels in the blood are linked to neurovascular problems in diabetic mellitus. MTHFR 677C/T (rs1801133) and 1298A/C (rs1801131), two frequently investigated polymorphisms, alter the structure and activity of enzymes by causing amino acid alterations 222Ala/Val and 429Glu/Ala, respectively. Following contradictory findings from many research, meta-analyses revealed a strong correlation with DPN[93,101,96,104]. MTHFR polymorphism has been shown to impact how the body reacts to a number of medications, including the diabetes medication Metformin. Patients with diabetes mellitus who are on therapy and have dangerous MTHFR genotypes should take vitamin supplements since using metformin might cause vitamin B12 deficiency [93].

NOS3 (eNOS) preserves endothelial cell function and homeostasis while controlling NO circulation levels by producing NO from L-arginine. The NOS3 gene harbors three noteworthy variations: the functional SNP 894G/T (rs1799983), the promoter variant-786T/C (rs2070744), and the variation in the 4a/b tandem repeats in intron 4. Numerous studies have demonstrated a connection between the beginning and development of DN leading to DPN and polymorphisms of the all three NOS3 isoform [93,105,106]. Given that NOS3 is linked to inflammation which ultimately leads to neuropathic pain, and that the variations indicated may serve as pharmacogenetic indicators, it is an intriguing target for therapy[93].

A chemokine called VEGF controls angiogenesis, modifies vascular permeability, and favorably affects neurogenesis[93,101]. Two polymorphic sites associated with the VEGF gene, rs6921438 and rs10738760, account for almost half of the variance in VEGF levels. It has been shown that DPN and VEGF-related polymorphisms are associated. It is necessary to do more research to determine the role and pharmacogenetic implications of these genetic variations in DNs [93,101,107].

The relationship between the anti-oxidative mechanisms in DN and variations in the genes encoding universal enzymes that catalyze hydrogen peroxide removal or quench reactive compounds has been extensively studied. Meta-analyses revealed a correlation between DN and the SNPs 599C/T (rs1050450) in the GPx-1 (glutathione peroxidase 1) and -262C/T in the CAT (catalase) gene. However, variations in GSTM1 as well as in GSTMT1 (glutathione S-transferases M1 or T1) did not appear to be associated in any way [101108]. The topic of DN is a focus of current genomic research; Meng et al. reported the findings of a genome-wide association analysis carried out in Scotland. It found that the chromosomal regions 8p21.3 and 1p35.1, which were more common in women, were linked to neuropathic pain in DPN [109].

Table3: The gene polymorphism involved in diabetic Peripheral Neuropathy.

Gene Involved	Polymorphism	Risk Allele	Mechanism	References
ALR2	-106C/T	T	Polyol pathway, oxidative stress	[110]
ALR2	50-(CA)n	Z-2 (CA22)	Polyol pathway, oxidative stress	[111,112]
ACE	I/D (intron 16)	D	Inflammation Reactive oxygen species	[113,114]
APOE	e2, e3, e4	e4	Neuronal regeneration	[93,115]

MTHFR	677C/T	T	Metabolism of homocysteine and folate cycle	93,116,117
MTHFR	1298A/C	C	hyperhomocysteinemia, lipid metabolism	93,116118
NOS3	-786T/C	C	vascular changes, oxidative stress	93,119,119
NOS3	894G/T	T	vascular changes, oxidative stress	93,119120
NOS3	4a/b	4a	vascular changes, oxidative stress	93,120
6p21.1(VEGF-related)	rs6921438 A/G	A	Angiogenesis	93,116
9p24.2 (VEGF–related)	rs10738760 A/G	A	Blood vessel alteration	93,121
GPx-1	599C/T	T	oxidative stress	116,122
CAT	-262C/T	T	oxidative stress	116,122

The AR gene's participation as etiopathogenesis in individuals with DM may be related to the propensity for methylglyoxal (MGO), advanced glycation end product (AGE), and oxidative stress in a hyperglycemic condition to promote AR gene expression[123]. Other investigations on DN pathology in people and rodent models have not consistently shown that mice lose more nerve fibers, even over an extended period of time, in DM[123]. As a result, it is plausible to believe that additional variables associated in the development of DN, even though it has been proposed that AR may be engaged in the initial stages of DN[123].AR enzyme is encoded by the ALR2, whereas the SDH gene codes for the second enzyme, SD in the polyol pathway [124].

In Sivenius et al.'s study, patients with DM type 2, polymorphism at -106C/T of the ALR2 gene's promoter region which dysfunction the motor peroneal nerve's nerve conduction velocities and a decrease in amplitude of the sensory nerves when compared to subjects with the -106C/C genotype[125]. Heesom et al. discovered a connection between the DN and another polymorphism in the 5',upstream regulatory region of ALR2 when studying people with insulin-dependent diabetes. The 50-(CA)n microsatellite polymorphism is composed of more than ten alleles; Z-2 and Z+2, where Z stands for 24 CA repetitions, are the two most prevalent variants. The Z+2 allele was found to be much less common in the DN patient group[126], although Z-2 allele was found to be more common in individuals with both types of diabetes mellitus[123]. It should be noted that these markers are not found inside the AR gene's protein-coded domain, suggesting that they shouldn't have a direct impact on the structure and functionality of the enzyme[123]. Therefore, the transcription of the AR gene or increased expression of the gene might be the potential mechanisms of action for some of these genotypes[123]. Taking into account the role of SD in DN, it is noteworthy that nerve sorbitol levels in mice lacking SD do not indicate DN vulnerability[123].

The sodium channel Nav 1.7 is encoded by the SCN9A gene and is implicated in pain signaling, is one such example. This gene mutations have been seen in both painful DPN [127] and idiopathic small fiber neuropathy [128]. Rare Nav 1.7 variations were discovered in 10 out of 111 patients with painful-DPN in the Blesneac et al. research[129], whereas none of the 78 responders with

painless-DPN had them. Furthermore, those with Nav 1.7 mutations displayed more intense pain and a shorter duration of diabetes. From a therapeutic perspective, these findings are intriguing since carriers of these genetic variations who were treated with the anticonvulsant such as lacosamide was reported as better pain relief than those who received a placebo[130].

4.5 Proteins

4.5.1 Toll-like receptor (TLR)

The innate immune response depends on the toll-like receptor (TLR) family of proteins. Interactions between TLRs and their ligands generated by microbial pathogens can trigger an adaptive immune response and create cytokines[131]. TLR4 is one of the TLRs that is involved in a lot of inflammatory diseases. Research using animal models as well as human studies suggests that TLR4-mediated system inflammation has a role in the pathophysiological process of diabetes [132,133].

Important for host defense, TLR4 is an innate immune receptor that may identify pathogens, trigger an inflammatory response, and start an adaptive immunological response. The developing adipose tissue of db/db mice has been shown to produce more TLR4 mRNA[134], and there is a strong correlation between enhanced TLR4 expression and TLR-mediated inflammation in monocytes as well as elevated HbA1c levels in diabetic humans[132]. TLR4 genotypes Asp299Gly and/or Thr399Ile are associated with a decreased incidence of diabetic neuropathy in individuals with type 2 diabetes[135], and it has also been shown that TLR4 deletion in mice lowers the proinflammatory state of diabetes [136]. Taken as a whole, our results suggest that TLR4 may play a role in the pathogenesis of DPN and diabetes.

TLR4 activates downstream processes inside the cell, including the NF-kB pathway and mitogen-activated protein kinase (MAPK). Proinflammatory cytokines, such as TNF-α and IL-6, are synthesized and released when the NF-kB pathway is activated, but the control of neural plasticity is significantly impacted by the activation of the MAPK pathway[131]. Assays using peripheral blood mononuclear cells and inhibitors of IKK (inhibitory B kinase) pathways have shown the critical function of NF-kB in glucose metabolism, inflammatory responses, and insulin response [137]. TLR4 was further verified by OR, and receiver operating characteristics (ROC) analysis suggested that TLR4 had a substantial correlation with the presence of DPN. Elevated TLR4 levels are indicative of markedly elevated risks for diabetic neuropathy in both the general population and diabetic subjects[138].

4.5.2 Glycated Hemoglobin

As the primary risk factor for diabetic peripheral neuropathy (DPN), glycemic variability (also known as hemoglobin A1c or HbA1c) levels are monitored on a regular basis. Chronic sores, skin ulcerations, and a lack of protective feeling are the results of DPN progression. Despite the fact that several studies have proven the significance of DPN in the etiopathogenesis of foot ulcerations, a lack of awareness and incorrect management of DPN has resulted in a significant number of needless lower limb amputations[139]. When combined with diabetic neuropathy, hyperglycemia has an impact on keratinocyte and fibroblast activity, which may be crucial in the pathophysiology of diabetic foot problems and amputation [140141]. These two elements are closely associated because hyperglycemia affects chemotaxis, which leads to severely disrupted cell proliferation and migration healing process, and the pathophysiology of neuropathy initially includes undetected injuries within skin regions[140].

In diabetes mellitus type 1, axonal degeneration is mediated primarily by axonal ion channel failure, which is a component that is significantly influenced by glucose fluctuation[142]. Diabetic foot development may be aided by the chronic inflammatory demyelinating polyneuropathy

that patients with diabetes mellitus are more likely to acquire [143]. Intra-axonal Na+ buildup results from hyperglycemia, which causes an active polyol pathway to shunt extra glucose and impair neuronal Na+ /K+ -ATPase (Figure 1). The observed alteration pattern aligns with axonal depolarization, an anomaly that might arise when the energy-dependent axonal Na+/K+ pump is malfunctioning[144]. A characteristic that affects around 30% of diabetics and may be a sign of the illness itself is increased glucose, which can also have an impact on the skin[145].
The American Diabetes Association's guidelines state that the objective of type 2 diabetes medication is to lower glycated hemoglobin A1c (HbA1c) to 7% or 6.5%. HbA1c is a measure of glycemia over a two- to three-month period. High HbA1c levels are mostly linked to inadequate wound healing, and in diabetes individuals, HbA1c is a useful biomarker for foot ulcer outcomes (wound healing time)[146]. Elevated HbA1c measurements may serve as a useful biomarker for the early identification of diabetic peripheral neuropathy in the feet. In fact, reduced HbA1c levels and strict glycemic control are linked to a decline in diabetes complications: a decrease in HbA1c of less than 7% is linked to a 60% decrease in the risk of peripheral neuropathy[147,72]..

5. CONCLUSION

Diabetic peripheral neuropathy is becoming more and more common, approaching epidemic levels. We may assume that if DM frequency increases and changes in the body and diet result, a substantial percentage of patients will experience its chronic side effects, such as foot ulceration and DPN. There are currently no optimum biomarker and ideal endpoint available for the diagnosis of DPN. Therefore, this review focus on the biomarkers of nerve damage in order to diagnose DPN in patients and to assess potential treatments in clinical trials.

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