

**ABSTRACT**

Diabetic neuropathies comprise a constellation of clinical signs and symptoms, with changes affecting peripheral nerves, autonomic nerves and central nervous system. This may be associated with either diffuse or focal damage or degeneration. Virtually all organ systems maybe involved including diabetic foot, eye, cardiovascular, gastrointestinal and urogenital systems and maybe associated with other autonomic dysfunctions. Hyperglycemia is the core dysfunction, but how it leads to neuropathy maybe mediated by numerous mechanisms which may involve genetic predisposition, osmolyte accumulation, oxidative stress, ischemia, neurotropic factor deficiency and immunologic molecular interplay. These molecular mechanisms causing diabetic neuropathy are briefly discussed.

**KEY WORDS:** Diabetic neuropathy; Molecular mechanisms

**INTRODUCTION**

Diabetic neuropathies are common in clinical practice as a major cause of incapacitation, morbidity, devastating complications and premature death (13). These neuropathies comprise of clinical changes in peripheral nerves, autonomic nerves and central nervous system, with diffuse or focal damage/ degeneration. Diagnosis involves symptom profile scores, neurological examination, electrophysiologic nerve conduction study, quantitative sensory scores and exclusion of other potential causes of neuropathies (4). None of the diabetic neuropathic syndromes are specific or pathognomic for diabetes. Peripheral neuropathy is associated with axonal injury, demyelination and axoglial dysfunction. Neuropathic consequences predispose to ulcers (cellulitis, gangrene), ophthalmologic, cardiovascular, urogenital, gastrointestinal and other autonomic complications.

Diabetic neuropathy represents a dynamic flux between neuronal degeneration and regeneration. Major pathogenic mechanisms include: chronic hyperglycemia, insulin deficiency, osmolyte accumulation, oxidative stress, ischemia, neurotropic factors deficiency and immunologic

molecular interplay. These are all closely interrelated to induce cellular signal pathway alterations and gene expression of proteins. Immunohistochemistry with gene product protein (PGP9.5) of peripheral nerve axons, MRI imaging, positron emission tomography imaging of autonomic nerves, immunodiagnosis of neurotrophins and cytokines - offer greater scopes for accurate and early diagnosis in future (5).

Molecular biology has advanced rapidly in the last decade permitting characterization of molecular defects such as protein abnormalities by rDNA technology (cloning, epitopes, C-DNA expression), immunogenetics of cytokines (T cell helper types 1, 2 or both), nuclear factors (promoters, enhancers, DNA protein AP-1, NFAT) and transcription factors, using candidate genes or positional genetic probes. Some of this technology may come into widespread clinical use soon. Understanding the putative molecular signals will result in more effective therapies for diabetic neuropathy to retard degeneration, while enhancing nerve regeneration.

**MULTIFACTORIAL PATHOGENESIS**

Multiple pathogenic factors are interrelated in diabetic neuropathy. Hyperglycemia induced changes in polyol pathway causes accumulation of osmolytes (sorbitol, taurine, glycerophosphoryl choline, high aldose reductase), which reduce  $\text{Na}^+/\text{K}^+$  adenosine triphosphatase activity, resulting in  $\text{Na}^+$  retention, cellular edema and cell lysis (6). Local nerve ischemia induces thickened basement membrane, endothelial cell proliferation, vessel contractility anomalies, hypoxia and occlusion. The redox status of cell is reduced (NADPH, glutathione) whereas reactive oxygen species is increased (oxidative stress). Advanced glycation end (AGE) products promote auto-oxidation of glucose, endothelial changes, macrophage alterations, nitric oxide (NO) quenching and further increase free oxygen generation. Deficiency of GLA (dihomo'-r-linolenic acid) and n-acetyl carnitine with increased mitochondrial electron chain II complex, accelerate hyperglycemic damage. Microvascular insufficiency of endoneural and perineural vessels and decreased blood flow with loss of ionic charges, has been correlated with neuropathy.

## Protein Kinase C

Hyperglycemia increases diacylglycerol (glyceraldehyde-3-phosphate dehydrogenase) with activation of protein kinase C (PKC) (signal transduction system, PKC-B2 isoforms) and increases cPLA<sub>2</sub>, PGE<sub>2</sub> activity (7). Activated PKC modulates genetic mRNA expression of basement membrane matrix proteins (b FGF), glycosylation enzymes (CORE 2 G<sub>1</sub> cNA<sub>c</sub> transferase), contractile proteins (actin, myosin, caldesmon) and increases oxidative stress. Multiple changes in endothelium (ET), macrophages (TNF $\alpha$ , IL-1 ) and tissue activators (plasminogen inhibitor-1, fibronectin, laminin, thrombomodulin), lead to reductions of neurite growth and vascular elasticity. AGE products activate nuclear factors (NF Kappa  $\beta$ , AP-1), inflammatory cytokines (TNF $\alpha$ ) and stress responsive mitogen activated protein kinases (JNK--1, P38), which increase further production of reactive oxygen species and cause neuronal damage (8). Inhibitors of protein kinase C (bisindolylmalcimine, hispidin), alpha lipoic acid, electron transport chain uncouplers and other molecules are currently being investigated (9). AGE inhibitor (aminoguanidine), prevents these changes in pericytes and vascular endothelium, under experimental conditions. PKC pathways are also involved in vascular endothelial dysfunctions of diabetes by PKCB 2 translation from cytosol to membrane intracellularly and by modulating metabolic flux. Inhibitors of mitochondrial electron transport chain complex can prevent the hyperglycemia induced PKC changes (10).

## Neurotrophins

Insulin signaling resistance of neurons (Schwann cells, neurons, ganglia) with post receptor IRS-1 defects, may be associated with microtubular protein changes (tau proteins, presenilin PS-1, amyloid precursor protein) and angiotensin converting enzyme polymorphisms. Diabetic neuropathy is also characterized by ion channel dysfunction (Ca<sup>++</sup> flux, K<sup>+</sup> current) and delayed regeneration (high protein kinases, CDK5). IGF-I and II in neurons, promote nerve regeneration, astrocyte functions and co-ordinate with glial cell neurotrophic factor changes. IGF-I may promote neural regeneration.

Nerve growth factor (NGF) developmentally regulates neuronal growth and survival of neural crest cells, sympathetic and dorsal root ganglion cells. Neurotrophic factors diffuse into the sensory neurons and motor neurons and they are retrogradely transported from the nerve terminals to

the cell bodies. Neurotrophins (NT) promote development, survival and differentiation of neurons. Different tropomyosin related kinases (A, B, C) have been identified, which can modulate nerve functions. Circulating NGF levels are low in diabetic neuropathy. NGF deficiency reduces neurofilament gene expression and mRNA of substance P, with diverse alterations of vasodilatation, gut motility and nociception. NT<sub>3</sub> may modulate large fibre functions (11). However no direct correlation between availability of neurotrophins to pathogenesis of peripheral neuropathy has been established so far. Functional deficits in small nerve fibers have been correlated with neurotrophin deficiency. Other neurotrophins supporting sensory neurons, motor neurons and trophic neuronal families have been reported (NT<sub>3</sub>, 4, 5, 6 and trophomyosin related kinase 7, 8, p.75, glial cell derived NF). In diabetes, neurotrophin NGF deficiency is observed. Six months of NGF therapy (1 mg/Kg) has shown beneficial effects with minimal hyperalgesia (12).

## Immunologic Molecules

Perineuronal vasculitis with autoimmune lymphocytic infiltrates and lumbosacral plexopathy was described in 1984, which improved with corticosteroids or immunosuppression (13). Heavy infiltration with endoneurial T cells was prominent, with activated CD8<sup>\*\*</sup> and CD4<sup>\*\*</sup> populations. Autoantigens in neurons with local immunological specific T cell interactions, HLA class 2 macrophages generating IL-2 receptors, TNF $\alpha$  secretion, mediating autoimmune injury of neurons, has been identified, even though the initiating factors are not known. Circulating antineuronal antibodies, complement fixing deposits, anti GM<sub>1</sub> ganglioside antibodies (12%) and antiphospholipid antibodies (88%), in diabetic neuropathies, has been reported. Hyperphosphorylation of neurofilaments may delay neurodegeneration of fibers in diabetes (14).

## SUMMARY

Diabetic neuropathies are heterogeneous clinical and subclinical syndromes - quiet at onset. but damaging devastatingly, when progression occurs. Neurologic dysfunction is complex phenomena, involving tissue hypoxia. degeneration and insulin signaling resistance. Advanced glycation end products induced molecular changes, induce neuronal loss. Mutations of nuclear factors (PSI-1 presenilin), enzymes (glycogen synthetase K-3,B), protein kinase C (increased), activated cytokines, JAK Kinases and genetic polymorphisms, tissues

with decreased NO production, ion channel dysfunction and delayed neuronal regeneration (15) all contribute to evolution of neuropathy. Molecular inhibitors of aberrant signal pathways. may in future, alter the current management of diabetic neuropathies. Glycemic control, aldose reductase inhibitors, gangliosides, linoleic acid (GLA), AGE product inhibitors, n-acetyl carnitine, myoinositol, nerve growth factor, corticosteroids, immunoglobulin G, antioxidants, ACE antagonists, endothelin blockers and PDIE inhibitors, have additionally found use in amelioration of diabetic neuropathy.

## REFERENCES

1. Thomas. P K , Classification of diabetic neuropathy, Diabetes Care: 1997; 46, S54-7.
2. Pommand. R, Diabetic neuropathy, Neurol Clin, 1997, 15 568-76
3. Feldman, E L. Greene D.A., Pathogenesis of diabetic neuropathy. Clin Neuro Sci., 1997; 4: 365-70.
4. Kennedy W R. Crapp, G , Quantitation of epidermal nerves in diabetic neuropathy. Neurology, 1996; 47 : 1042-8
5. Vinik, A I. Diabetic neuropathy, Amer. J. Med., 1999 107. 175-265.
6. King. Brownlee. Complication of Diabetics, Endocrinol Metab Clin, 1997; 27 : 250-69.
7. Cameron N.E., Colter M.A., PKC. effects on nerve function in diabetic rats. Diabetologia, 1999; 42: 1120-30.
8. Guha, M., Bai, W., Natarajan. R., Molecular Mechanisms of TNF alpha gene expression, J. Biol Chem 2000; 275. 7728-39.
9. Gainnini, C. Dyck, T.J., Basement Membrane reduplication and pericyte degeneration. Ann. Neurol. 37 1995; 498-504
10. Nishikawa T., Edelstein D. et al., Mitochondrial superoxide dismutase and its role in causation of hyperglycemic damage, Nature 2000; 404: 787-90.
11. Anand, P., Terenghi, G., Warner, G. Endogenous nerve growth factor in human diabetic neuropathy. Nat. Med., 1996, 2 : 703-7.
12. Apfel S.C., Kessler J.A., r human NGF in treatment of diabetic polyneuropathy, Neurology, 1998: 51 : 695-702.
13. Krendel, D.A., Younger D.S., Autoimmune Diabetic neuropathy, Neurol Clin. 1997; 15, 959-72
14. Kishi Y., Low, P.A., Alpha lipoic acid in experimental diabetic neuropathy, Diabetes, 1999; 48, 2045-51.
15. Yuar, S.Y. et al., PKC activation in diabetes, Circ Res, 2000; B: 87; 412-7.