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AN INSIGHT TO THE BIOMARKERS AND POTENTIAL NEW DIAGNOSTIC TOOL FOR DIABETIC NEUROPATHY

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ABSTRACT

Diabetes is an increasing epidemic worldwide and associated diabetic neuropathy is its most common disabling complication. Despite the global prevalence and severe complication of diabetic mellitus, the pathophysiological mechanism of diabetic neuropathy has not been elucidated. As diabetic neuropathy has different clinical manifestations it is very difficult to get early detected. Early diagnosis is recommended and is a key factor for the effective prognosis and prevention of further complications. However, diagnostic tests for diabetic neuropathy are not accurately established due to numerous pathologies developing from the nerve injuries and different mechanisms due to diabetes. So, biomarker is the key area for the research which can extensively useful for early detection of disease, monitoring of its severity and progression as well as to design accurate treatment regimen. We have reviewed available biomarkers which are used in diabetic neuropathy studies along with some potential new biomarkers which can be useful for the early detection of diabetic neuropathy.

KEYWORDS: Diabetes, Neuropathy, Biomarkers, diagnostic tools.

INTRODUCTION

Diabetes, a metabolic disease characterized by chronic hyperglycemia and is categorised as Type 1 (A chronic condition in which the pancreas produces little or no insulin), Type 2 (A chronic condition that affects the way the body processes blood glucose). In this body is in continuous state of hyperglycemia and this long term hyperglycemia damages the vasculature, nervous system and leads to downstream metabolic dysfunction. It majorly

affects renal function (nephropathy), sensory neurons (neuropathy), vision(retinopathy) and macrovascular events such as heart attack and stroke.[1]

Diabetic neuropathy is defined as neuropathic disorder that affects all types of peripheral nerves, including sensory, motor and autonomic nerves thus affecting nearly all body organs and system but usually most affected nerves are long somatosensory nerves of hands and feet leading to either loss of sensation or severe pain. This damage to long nerve fibre is believed to happen due to dysfunction of small nerve fibres. Small peripheral nerve fibre generally constitutes of 70-90% of peripheral nerve fibres. They have some distinctive functions such as tissue blood flow, temperature and pain perception and sweat production. Many studies have shown that small fibre damage might lead to large fibre damage and then too in neuropathy. Apart from this nerve damage there are several other pathogenesis of systemic and cellular disturbances in glucose and lipid metabolism leads to other complex biochemical pathways ultimately leading to neuropathy.[2]

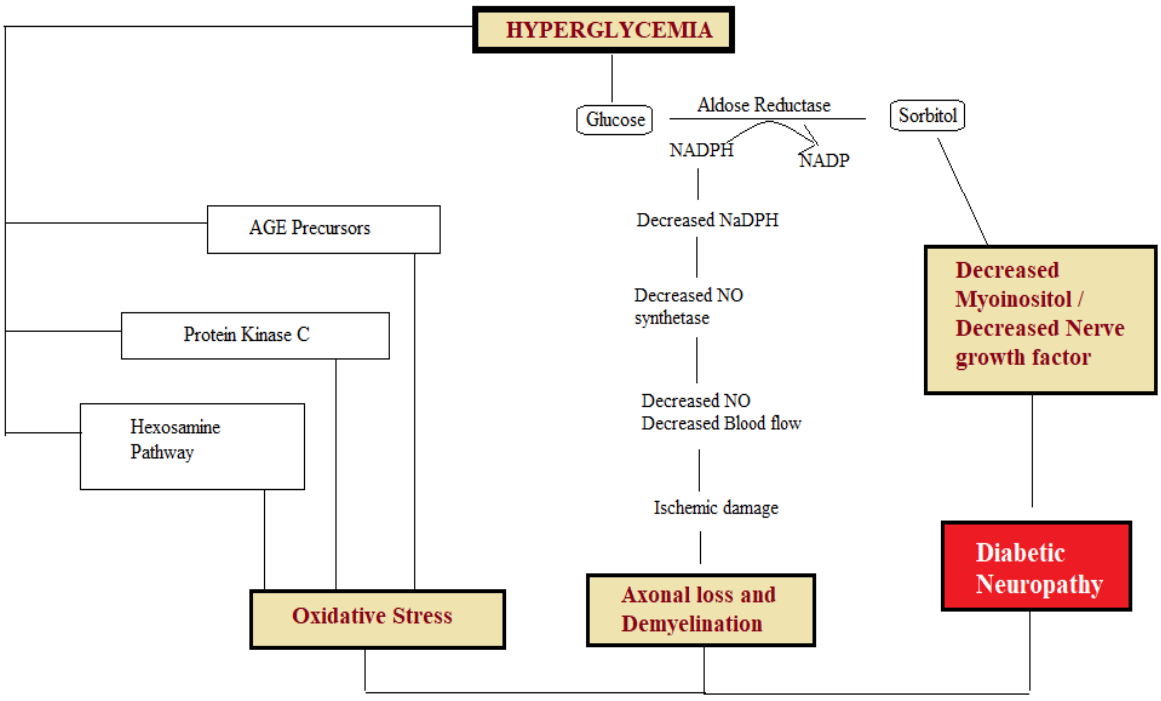

Figure 1: Pathogenesis of Diabetic Neuropathy.

So, it is important to know about the different biomarkers of neuropathy for the early diagnosis of condition and to manage the condition with proper and effective way. Though extensive research has been done in this area although an appropriate and widely utilizable biomarker has not been identified for neuropathy.

The aim of this review is to highlight the available biomarkers of diabetic neuropathy along with tools and methods to investigate it, also some potential new biomarkers which will be useful for the early detection of diabetic neuropathy are listed.

BIOMARKERS OF DIABETIC NEUROPATHY

Identification of sensitive biomarker is essential for the investigation of presence, severity and progression of the disease. No reliable biomarker of the progression of diabetic neuropathy is currently available. Some of the biomarkers which are frequently used in the research studies of neuropathy are molecular, structural, functional and behavioural biomarkers.

Molecular Biomarkers

Although hyperglycemia is the main cause of neuropathy, biochemical alterations that occurs during diabetes mellitus interferes with many complex processes leading to up-regulation or down-regulations of many molecules. The products of this alterations eventually lead worsening of condition therefore study of such molecules is important. Treatment of diabetic neuropathy does not only contain maintenance of glycaemic levels but also contains healing of the degenerated neurons. levels of certain proteins and peptides are fluctuated in the degeneration of neurons so, monitoring level of such markers are important to understand the stage and progress of the disease.

Molecular biomarkers of neuropathy reported in diabetes are as follows.

a) Down-regulation of neurotrophic factor especially nerve growth factor.

The goal in treating diabetic neuropathy is not only to prevent the progression of neuropathic symptoms and nerve dysfunction and degeneration but also to promote regeneration of degenerated nerve fibres. Bio-synthesis capacity of neuron mainly depends upon the messenger RNA (mRNA)in the nucleus also the need translocation of macromolecules and organelles from site of synthesis to axonal extremities. so, difficulties in the transportation of macromolecules is consider as one of the etiologies of diabetic neuropathy.[3] Nerve growth factor is one of the neurotropic factor which is responsible for maintenance and fine-tuning expression of neurons. Many studies have reported that there is decreased capture and retrograde transport of NFG in diabetic conditions. Increasing duration of hyperglycemia leads to reduction in NGF mRNA in different tissue.

Methods to investigate levels of NGF.

- Capture and retrograde transport of NGF in sciatic nerve is checked by injecting iodine labelled NFG in the footpad of rats.
- levels of NGF in tissues are investigated by performing assay on respective homogenate of the tissue.[4]

In addition to this other molecular biomarkers which affects the nerve regeneration and are down-regulated in diabetic neuropathy are as follows.

- Growth- associated protein-43 (GAP-43)
- Tα1α-tubulin
- Brain-derived neurotrophic factor (BDNF)
- Neurotrophin-3
- Neurotrophin-4/5
- Insulin like growth factor Ⅰ / Ⅱ
- Insulin like growth factor-binding protein (IGFBP-3) [3]

b) Biomarkers of oxidative stress

Normally in primary DRG neuron and axon, high glucose and excess fatty acids are used to produce the electron donors NADH and flavin adenine dinucleotide ($FADH_2$). complex Ⅰ to Ⅳ shuttled this electron donors through oxidative phosphorylation till they are donated to molecular oxygen to form water molecule. This electron transfer is important for ATP generation which is crucial for the mitochondrial viability, function and normal neuronal metabolism. As this processes, reactive oxygen species are produced while transfer of electron from complex Ⅱ to usually they are very small amount of biproduct and immediate neutralised in the nerve by cellular antioxidant such as glutathione, catalase and superoxide dismutase.[5]

Increased duration of diabetic mellitus leads to excess metabolism of substrate through glycolytic, β oxidation and TCA cycle there by increasing the NADH and $FADH_2$ electron donors in neuron. This disrupts the normal gradient, it affects oxidative phosphorylation in turns decreasing ATP production but increasing production of reactive oxygen species.[6] The cellular antioxidant system gets unable to neutralise this over reactive oxidative species and hence this unnaturalised ROS initiate the severe injury cascade which is worsened by loss of ATP leading to neuronal dysfunction.

Markers of oxidative stress are as follows.

- Malondialdehyde (MDA) and nitric oxide (NO) are the markers of oxidative stress.

Their levels in tissue are investigated by performing respective assays in tissue homogenate.

- Glutathione (GSH), Glutathione reductase (GR), catalase, superoxide dismutase (SOD) level are decreased in diabetic conditions, as they are members of body's antioxidant system.

Their levels are also investigated by performing respective assays in tissue homogenate.

c) Biomarkers of inflammation

Inflammation is one of the pathway in the development and pathogenesis of diabetic neuropathy. Systemic and cellular imbalances in glucose and lipid metabolism leads to various biological pathways including oxidative/nitrosative stress, activation of polyol and protein kinase C, cyclooxygenase 2 activation, endoplasmic reticulum stress and low-grade inflammation. This trigger the process of cytokine and chemokine production, including pro inflammatory interleukins and tumour necrosis factors.[7]

Markers of inflammation are as follows

- Interleukine-1 (IL-1)
- Interleukin – 6 (IL-6)
- Tumour necrosis factor α (TNFα)

Structural Biomarkers

Molecular changes and changes in biochemical pathways eventually lead to the alteration of cell organelles their by causing structural damage. If the structural pattern is studied properly little bit changes in it can be used to diagnose or stage the diabetic neuropathies.

a) Epidermal nerve fiber quantification

Skin biopsies are valuable means of diagnosis and staging of the diabetic neuropathy. This is minimally invasive technique which can differentiate between the types of fibers such as small unmyelinated fibers which are difficult to evaluate by other means. The skin consists of low threshold mechanoreceptors, thermoreceptors and nociceptors along with their myelinated and unmyelinated axons. This fibers starts from dorsal root ganglion of the neuron to the stratum Basale of the epidermis. This epidermal nerve fibers cane be divide into 2 sets, peptidergic and non-peptidergic.

To quantify the epidermal nerve fibers antibodies are developed against variety of neuronal marker proteins which allows immunohistological assessment.[8] Antibody against Protein gene product 9.5(PGP9.5), a cytosolic ubiquitin hydrolase is most commonly used as this protein is found in all neurons. Antibodies against neuropeptides such as calcitonin gene-related peptide (CGRP), substance P are also been used for selectively identify peptidergic nerve fibers. This immunoreactive profiles can be visualised using conventional light microscopy, fluorescence microscopy or confocal microscopies.[9]

b) Axonal number and calibre

It gives information about morphological changes in diabetic conditions. Studies have shown that hyperglycemia leads to smaller mean fiber, thinner myelin sheath and reduction in fiber density and number.[10]

Respective staining methods are used to perform this histological methods.

c) Axonal width

Diabetic neuropathy affects both large and small sensory nerve fibers. Most of the research focuses on small fiber neuropathy which deals with pain sensations. The little studies large fibers are also important as they are related to postural control, gait and balance. Muscle spindle are involved in sensory motor behaviour. Many studies have shown that damaged to muscle spindle leads to alteration in axonal morphology.

Muscle spindle quantification is done by using immunohistochemistry and fluorescent imaging, and from this spindle images axonal width is calculated.[11]

Behavioural markers

Signs and symptoms of sensory dysfunction, such as sensory loss, spontaneous pain, paraesthesia and allodynia are mostly the earliest and most evident manifestation of diabetic neuropathy. Behavioural markers include neuropathic pain which is characterized by mechanical/chemical hyperalgesia, tactile allodynia in sensory large fibres, thermal nociception in sensory small fibres and sensory motor deficit in large sensory fibres. Behavioural tests commonly employed should be presented and interpreted as measurement of combined sensory/central nervous system/motor functions and not solely as measurement of pain or sensory function.[12]

a) Neuropathic pain

Chronic hyperglycemic condition leads to alteration in nerve fibers. It is observed that strong spontaneous pain is seen in diabetic rodents which are otherwise not seen in healthy rodents. Along with this animals shows pain associated behaviours such as depression, audible or ultrasonic vocalization, limb guarding or autotomy. Hence this changes can be used as behavioural responses to sensory stimuli to indicate sensory dysfunction in diabetic patients. The major behavioural parameter is pain which is allodynic in nature that is elicitation of pain by such stimulus which normally does not considered as noxious such as light, touch, pressure and mild temperature change which causes hyperalgesia and pain to loss of pain sensation.

Many of the tests are applied to induce pain and majorly they are categorised as stimuli provided by temperature, pressure or chemical agent which are applied to paw or tail of rodents. This methods are advantageous because animal restraining is not required, the rate of heating, pressure or amount of chemical causing stimuli can be varied according to stimulation of different nerve fiber type. But sometimes the results are affected due to changes in skin thickness and collagen modification.[12]

There is changes along the duration of hyperglycemic state. Short term diabetic is prone to hyperalgesia to stimuli like chemical, pressure or temperature whereas as the duration of disease increases the thermal and mechanical hypoalgesia's are seen. So, this pain parameter is useful for staging of the disease.

Table 1: Tests to investigate neuropathic pain.

Method	Test
Thermal Method	1. Tail immersion test 2. Hot plate method
Mechanical Method	1. Van Frey test 2. Paw pressure test
Chemical Method	1. Injection of formalin into paw - High concentration (0.5-5%) - Low concentration (0.2%)

b) Motor Coordination

The sensorimotor deficit resulting from large motor damage can leads to significant impairment. Which can cause deficits in lower limb proprioception, decreased tactile sensitivity and vibrational sense and incoordination due to balance abnormality.[11] Various

studies has shown that patients with diabetic neuropathy are at an increased risk to fall due to decreased postural control and altered gait and balance.[13]

The underlying cause of large fiber neuropathy is not fully understood but it has been hypnotised that it is caused due to specific damage to group Ia and II sensory afferent fiber in muscle spindle. This muscle fibers are found in skeletal muscles and does rapidly adapt to sensorimotor receptors. Muscle spindles are involved in many sensorimotor behaviours such as the regulation of proprioception, balance, gait and the postural response, and spindle damage can lead to deficits such as motor incoordination.[11]

Methods used to investigate motor coordination are as follows.

1. Beam walk method
2. Foot printing method
3. Grid walk method
4. Rotarod apparatus
5. Walking track analysis
6. Gait analysis[14]

Functional markers

The alteration in the molecular pathways and structural component leads to disturbances in the function of the nerves which in turn cause conditions like altered conduction velocity in neurons, reduced blood flow etc which causes abnormalities in normal body mechanisms.

Functional biomarkers reported in diabetes are as follows

a) nerve conduction velocity

Electrophysiological study of nerve function in diabetes provide the information about nerve integrity as well as onset and progression of disease. As chronic hyperglycemia causes formation of superoxides. Along with oxidative stress they tend to get accumulate in endothelial and epineuria vessels which further causes nerve damage by damaging myelin sheath and causing nerve ischemia. Because of this both vascular and non-vascular reasons induction of nerve dysfunction takes place slowing the nerve conduction velocities in both sensory and motor neurons. Conduction study is both reliable and reproducible study hence it is useful in measuring nerve dysfunction. Small fibre neuropathy represents one of the earliest injury in diabetic neuropathy hence this conduction study qualifies as earliest diagnostic test for diabetic neuropathy.[12]

Study of nerve conduction velocity is non-invasive electrophysiological study.

It is performed by stimulating sciatic nerve. Then evoked potential is recorded from adjacent muscle then NCV is calculated by subtracting the distal from the proximal latency from the stimulus artefact of the take-off of the evoked potential, and the difference is divided into the distance between the two stimulating electrodes.[15]

b) Reduced nerve blood flow

Reduction in blood flow is one of the reason behind slow conduction velocities in nerves. Reduced blood flow leads to stage of ischemia in nerves their by causing nerve damage. Though nerves are resistant to ischemia, they do not need moment-to-moment supply of energy to carry their function. But in case of hyperglycemia there is increase in the metabolic need in nerves which is not fulfilled by coming amount of blood. Along with this high blood glucose leads to glucose loading which interfere with osmotic effect which end up in increasing blood viscosity their by reducing blood volume.[16]

Altered endothelium-dependent vasodilation is one of the reason for reduction in nerve blood flow. There are studies which show that Poly (ADP-ribose) Polymerase (PARP) overactivation is responsible for endothelial vasodilation dysfunction which results in nerve blood flow abnormalities.[17]

Various techniques for the investigation of nerve blood flow are as follows

- H_2 Clearance technique[18]
- Laser doppler flowmetry[19]
- Radiolabelled idoantipyrine technique[19]

c) Delayed gastric emptying, colonic and intestinal transit

Many gastrointestinal symptoms are seen in diabetic patients because of diabetic autonomic neuropathy. Among this symptoms gastroparesis is most common. Gastrointestinal motility disturbances including esophageal motor dysfunction, gastroparesis, constipation and diarrhea, are common in patients with diabetes mellitus. This all occurs because mucosal layer of gastrointestinal track is very sensitive to reactive oxygen species and oxidative stress. In case of chronic hyperglycemia there is excess oxidative metabolism of glucose and lipids causing excess production of free radicles which in turn causes damage to cellular macromolecular component like mitochondria, endoplasmic reticulum, protein etc. which

eventually leads to cell death. Along with this recent studies have shown that increase in reactive carbonyl compound derived from oxidative and non-oxidative reactions initiate chemical modifications in proteins which ends up into overpowering of oxidative stress their by causing tissue damage. This carbonyl compounds are believed to be neutralised by enzymes like glyoxalase pathway and aldose reductase, but in case of hyperglycemic condition level of this enzymes are reduced. hence body is unable to neutralise the carbonyl radicles which are responsible for carbonyl stress and consecutive oxidative stress.[20]

This gastro intestinal symptoms are clinically important because the associated with alteration in glycemic control and in oral drug absorption.[21] Markers of this gastrointestinal complications are delayed gastric emptying, intestinal and colonic transit. This all is alleged to happen due to damage of vagal and peripheral cholinergic neurons.[22]

Methods used to investigate delayed in gastric emptying, intestinal and colonic transit are as follows.

- Delayed gastric emptying, small intestinal and colonic transit of the phenol red meal.[23]
- Intestinal transit of charcoal meal can be determined by modified Janseen method.[22]

EARLY DIAGNOSTIC TOOLS FOR DIABETIC NEUROPATHY

Though extensive research has been done in the area of diabetic neuropathy, but lack of early diagnostic test and gold standard biomarkers are still lacking. These are potential biomarkers for the early diagnosis of diabetic neuropathy.

1. Skin's rewarming rate

Diabetic mellites leads to secondary disease such as microvascular dysfunction. Reduction in nerve conduction velocity is major complication which reported to precede due to impaired vasodilation because of microvascular dysfunction. Thermography study have shown decrease in the skin temperature in diabetic patients. So, some studies have demonstrated that the rewarming rate after cooling can be useful for diabetic peripheral neuropathy. As microvascular dysfunction is going on throughout the state of hyperglycemia therefore monitoring changes in skin warming rates may enable early diagnosis and management of diabetic neuropathy. To perform this experiment diabetic neuropathy was induced by single intraperitoneal injection of streptozotocin (STZ).Blood flow rate and oxygenation in the plantar hind paws of rats were measured non-invasively by combined laser doppler flowmetry. The temperature of skin was monitored by using in-built infrared digital video

camera. The skin temperature of entire plantar hind paw was recorded while the animal was fixed after placing the animal on 14 ◌C plate for 5 seconds. The average rewarming rate is demonstrated as increase in skin temperature per 120 seconds. Plantar skin of hind paws was dissected and immunohistochemical tests were performed to quantify the density of sensory nerve fibres innervating the skin.[24] The observations show that till 4 weeks oxygenation was well within the range but as the hyperglycemia precedes oxygenation was significantly reduced than the normal limits. This indicated severe macrovascular dysfunction. The difference in temperature between diabetic and non-diabetic rats became significant at earlier time points after cooling, 120 seconds after cooling in rats 4 weeks post induction, 90 seconds after cooling in rats 6 weeks post induction, 30 seconds in rat 8-week post induction. This indicates progressively increased delay in rewarming at later time points after induction of diabetes.

2. Neuron Specific Enolase (NSE)

A timely and accurate diagnosis of diabetic neuropathy is essential to early intervention for decreasing the rate of associated disabilities and death. Though many biomarkers are used for this diagnosis still biomarker related to specifically neuronal damage is still not known. Enolase are intracellular enzymes normally located in the cytoplasm of neuroendocrine cells. principally located in neuronal tissue, it is radially released into cerebrospinal fluid and blood with biological half-life of 48 hours. In the periphery of body nerves are bundled around the blood vessels supplying the organ. Surrounding this each fibre in periphery nerves is endoneurium which is analogues to blood-brain-barrier. So endoneurial fluid is like the cerebrospinal fluids. In case of this neuronal tissue damage the amount of this endoneurial fluid may increase due to irritation or noxious stimuli. But unlike the central nervous system which have protection of blood-brain-barrier the peripheral nervous system is more unprotected and readily affected by the stress. Chronic hyperglycemia leads to oxidative stress this inactivates many glycolytic enzymes including enolase. To fulfil the high energy requirement in case of oxidative stress the glycolytic enzymes are upregulated to avoid neuronal damage. During this the rate of synthesis of enolase in the damaged neuron is changed and is likely to cause NSE to leak in endoneurial fluid and serum. So NSE level in circulation may be an early indication of diabetic neuropathy.[25]

NSE levels are checked by using electrochemiluminescence immunoassay. There were only slight increased in NSE level with diabetic patients, but an increased level of NSE were seen

in patients with diabetic neuropathy and diabetic retinopathy. Further NSE levels increase with progression in stages of diabetic neuropathy from asymptomatic to the disability. Therefore, elevated levels of NSE indicates neuropathy with involved degree of nerve fibre damage which are associated with changes in synthesis and release of enolase. During the pathological changes such as demyelination and remyelination associated with neuropathy. As NSE was detected both in oligodendrocytes and neurons it indicates that NSE is released from not only affected neurons but also from affected Schwann cells which ultimately form myelin sheath. As the experiments proceeds it was realised that NSE levels associated with neuropathy is independent of other variables.[25] Increase in serum NSE can be used as indicator of diabetic neuropathy which will be helpful in the timely prediction, diagnosis and accurate treatment of diabetic population.

3. Calprotectin

Calprotectin is stable heterodimer belonging to S100 protein family, expressed in activated human granulocyte and macrophages in inflammatory conditions. Its functions consist of activation of NADPH oxidase, toll like receptor 4 (TLR4) and advanced glycation end products (AGEs)receptors. Among this AGEs receptor activation is one of the important pathway leading to micro and macrovascular complications in diabetes. This receptors interact with certain members of S100 proteins and implicate inflammatory disorders. Also, Nuclear factor-ƙB (NF-ƙB) is a transcription factor involved in many immune and inflammatory responses. Toll like receptors are responsible for activation of NF-ƙB and there by activating inflammatory responses. Calprotectin has also been identified as endogenous activator of TLR4. So, this study shows that, high levels of calprotectin detected in patients of diabetic neuropathy suggest that this molecule possess some pathogenesis in the neuroinflammation.[26]

The level of calprotectin in blood are measured by using specific Enzyme-linked immunosorbent assay detection kit (ELISA).

So, calprotectin which is critically involved in proinflammation can be a biomarker which will be beneficial in monetarizing disease activity.[26]

4. Heat Shock Protein 27 (Hsp27)

Heat shock protein 27 (Hsp 27) is a small protein known to protect cell apoptosis under stress. In experiments of nephrotic syndromes an increased expression and phosphorylation

of Hsp 27 is been seen indicating its upregulation in stressful conditions. Reports have shown that Hsp 27 is not restricted to the tissue but is also secreted into the circulation when in excess. Plasma levels of Hsp 27 are used as potential index in atherosclerosis experimentally.[27] Also serum levels of Hsp 27 are used in various diseases like abdominal aortic aneurysm, peripheral artery disease, poly neuropathy, Type 1 diabetes mellitus and chronic kidney disease. In the stressful condition which is created by heperglycemis Hsp 27 is upregulated to act as molecular chaperone. This upregulation is necessary for the survival of injured motor neurones.[28] Interestingly Hsp 27 was only found to significantly increase in case of neuropathy when compared with retinopathy, microalbuminuria or cardiovascular diseases. Thus, in case of diabetic complications Hsp 27 can be used as a selective marker.[12] Levels of Hsp27 are measured using respective ELISA kit.

5. Corneal Confocal Microscopy

Corneal Confocal Microscopy (CCM) is a new, Non-invasive, and reproducible diagnostic test. The cornea of eye contains numerous nerve fibers originating from trigeminal nerve and are mainly categorised in 3 groups and they are :

1) Sub basal plexus
2) Sub epithelial plexus
3) Stromal nerves

Among there the sub basal plexus underlying the basal epithelium is most important and sensitive to CCM for detection of diabetic neuropathy.

The technique basically uses a light beam which passes through an opening and appropriate focused by an objective lens onto the corneal layer which is to be examined.[30] Meanwhile all the light approaching from other sources is eliminated by beam splitter and photodetector devise. Using this basic setup 3 methods has been developed.

1) Tandem scanning CCM (TSC)
2) Slit scanning CCM (SSCM)
3) Laser Scanning CCM (LSCM)

SSCM and LSCM are the most useful in the case of detecting diabetic neuropathy. The LSCM uses a laser beam as a light source and provide high resolution and clear visualization of corneal epithelial and stroma.

The parameters which are assessed of corneal nerve pathology are:

1 Corneal nerve fiber density (CNFD) [Total number of major nerves per mm^2]

2 Corneal nerve fiber length (CNFL)

3 Corneal nerve branch density (CNBD)

4 Corneal nerve fiber tortuosity

Corneal nerve fiber pathology is severe in presence of diabetic neuropathy. CCM is sensitive enough to detect corneal nerve perturbation as early as possible. CCM parameters deteriotes progressively with increasing severity of neuropathy which is useful for staging the disease. It is observed that CCM can objectify early nerve fiber improvements. It has been experimentally seen that improved cholesterol levels after 24 months were linked with significant improvements in CNFD, CNBD and glycated haemoglobin reduction was significantly corelated with improved CNFD. Hence CCM can also be useful in monitoring effects of neuroprotective agents on peripheral nerve system.[29]

Table 2: Potential new biomarkers are classified as.

Biomarkers	Examples
Molecular	a) Calprotectin b) Heat shock protein 27 (Hsp27) c) Neuron specific enolase (NSE)
Structural	Corneal confocal microscopy
Functional	Skin rewarming rate

CONCLUSION

Neuropathy is one of the common complication of diabetes which have more than one pathologies. Hence appropriate biomarkers responsible should be known properly. Effective diagnosis and staging of disease can be done when accurate biomarker and clinical responses are known. Early diagnosis is critical for the successful management of diabetic neuropathy. Hence research in emerging new methods and their cost effectiveness for diagnosis are encouraged.

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