1. INTRODUCTION

1.1. Neurodegeneration and neurodegenerative disorders

“Neurodegeneration” is the umbrella term for the progressive loss of structure or function of neurons, including death of neurons. Neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease (PD), Huntington's disease, amyotrophic lateral sclerosis and prion diseases are increasingly being realized to have common cellular and molecular mechanisms including protein aggregation and inclusion body formation. Although each disease has distinctive morphological and biochemical characteristic the pathology of each is consistent with oxidative damage.

As we enter the next decade, the “new revelation” strikes where there will be increase in age-associated diseases including the most devastating of these, which involve the nervous system e.g. Alzheimer’s disease and PD. Common components thought to contribute to the manifestation of these disorders and normal age-related declines in brain performance are increased susceptibility to long-term effects of oxidative stress and inflammatory insults. Unless some means are found to reduce these age-related decrements in neuronal function, health care costs will continue to rise exponentially (Youdim and Joseph, 2001). With an increasingly aged population, neurodegenerative diseases will assume greater predominance, not least the disease described so beautifully by James Parkinson in 1817.

**Parkinson’s disease (Known in India as “Kampa Vata”)**

PD is the second most common neurodegenerative disorder affecting 1-3% of individuals over the age of 65 years that often impairs the sufferer's motor skills, speech, and other functions. The disease is named after English apothecary James Parkinson, who made a detailed description of the disease in his essay: "An Essay on the Shaking Palsy".
It is a slow progressive neurological disorder; in which there is wide spread destruction of substantia nigra (pars compacta) SNpc, which sends dopamine secreting fibers to the striatum. "Parkinson's disease" is the most common form of parkinsonism, and refers to the normal presentation of idiopathic parkinsonism’s (Jankovic, 2008; Poewe and Wenning, 2002) (Fig.1.1).

Genetic forms are usually included although the terms familial Parkinson’s disease and familial Parkinsonism are also used for disease entities with an autosomal dominant or recessive pattern of inheritance (Samii et al., 2004)

**Figure 1.1: Sagittal section of brain**

The primary symptoms are the results of decreased stimulation of the motor cortex by the basal ganglia, normally caused by the insufficient formation and action of dopamine produced in the dopaminergic neurons of the midbrain (specifically the substantia nigra). Clinical presentations in PD generally occur at the average age of 55-60 yrs and are divided in 2 types, motor (primary) symptoms and non-motor (secondary) symptoms. The former includes tremor, rigidity, bradykinesia, postural instability, gait, festination, dystonia, hypophonia, micrographia and dysphagia. Secondary symptoms are mood, cognitive, sleep, sensational disturbances, weight loss, incontinence and constipation (More and Sharma, 2006).

While effective therapy exists for treating the bradykinesia, rigidity and tremor associated with the disease, the cause is unknown. There is no treatment available to prevent or slow the progressive neuronal loss in the substantia nigra and associated decreased levels of dopamine in the striatum that underlie the cardinal features of the disease.

The current theory (so-called Braak’s hypothesis) is that the earliest signs of Parkinson’s are found in the enteric nervous system, the medulla and in particular, the olfactory bulb, which controls your sense of smell (Braak et al., 2003). Under this
theory, Parkinson’s only progresses to the substantia nigra (SN) and cortex over the years. This theory is increasingly borne out by evidence that non-motor symptoms, such as a loss of sense of smell, hyposmia, sleep disorders and constipation may precede the motor features of the disease by several years.

1.2. Epidemiology including prevalence, socioeconomic consequences, incidences and overall health costs

PD is one of the fatal and major neurodegenerative disorders of the modern society. It is a multifactorial disorder also called as “silent epidemic” because of its aggressive onset. Worldwide 4-6 million peoples are affected with over 1.7 million affected in China and 2.8 million in U.S.A (Benjamin and Joseph, 2001) (Fig.1.2)

Epidemiological studies have shown that the incidence of neurodegenerative disorders is generally lower in women as compared with men, and in the case of Parkinson’s disease, the risk/incidence of disease in men is twice that of women (Baldereschi et al., 2000; Behari et al., 2001; Eeden et al., 2003). (Fig.1.3).

Figure 1.2: Incidences of PD

PD affects the quality and quantity life of the patients, creates enormous burden on patients and their family, affects the normal day-to-day activity of a person and creates a serious economic drain on the society. Costs of treating PD in India are lower than those in developed nations but are still out of reach for most Indian patients. Nearly 16% to 41.7% of the average Indian gross annual income (GNI) has been estimated (considering GNI less than rupees 50,000) (Ragothaman et al., 2006).
1.3. Etiology and Pathogenesis

The transverse section (T.S) of brain shows the dark pigmented regions of substantia nigra in the midbrain region (Fig.1.4). The central nervous system has a high rate of oxidative metabolism relative to other tissues, receiving almost 15% of the cardiac output and accounting for up to 30% of the resting metabolic rate (Sokoloff et al., 1977).

Figure 1.4: T.S of brain showing basal ganglia and substantia nigra

In light of this, there is no wonder it exhibits increased vulnerability with age from the cumulative effects of oxidative damage to proteins, lipids and nucleotides (Harnam, 1956). Such oxidative damage has been observed in aged brains of various mammals. The pathological hallmark of adult-onset PD is the Lewy body, an inclusion body found in the cytoplasm of neurons, often near the nucleus (Fig.1.5).

![Figure 1.5: Positive alpha-synuclein staining of a Lewy body](image)

Lewy bodies are densest in the substantial nigra but can also be present in monoaminergic, cerebral cortical and other neurons. A major constituent of Lewy bodies is aggregated α-synuclein protein and can be labeled for ubiquitin, a synuclein interactor termed synphilin-1, proteasome proteins, and other proteins (Ross and Poirier, 2006).

Some of the factors involved in its etiology:-

1. Genetics: DJ1 and PINK1 are mitochondrial proteins and overexpression of α-synuclein and parkin (E3-Ligase) induces mitochondrial defects by impairing ubiquitin proteosome system (UPS) system.

2. Environmental risk factors- 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)- induced dopaminergic cell death is one of the major causes of PD. Use of pesticide and herbicide like Paraquat and Rotenone accelerates α- synuclein fibril
formation and lewy body inclusion causing irreversible nigrostriatal degeneration. Heavy metals exposure has also been proposed to increase the risk of PD.

3. **Excitotoxicity**: It indicates the injury to the brain caused due to excessive glutamate in the brain. Overloading of calcium stores excessively impairs and disrupts mitochondrial membrane, and causes decrease in ATP synthesis with generation of reactive oxygen species (ROS).

4. **Apoptosis**: It is called as cell suicide/ programmed cell death. Evidence of apoptosis in PD is DNA fragmentation, chromatin condensation in the same cell. Genes facilitating apoptosis include Bcl-2 and Bcl-xL and caspases.

5. **Oxidative stress**: In PD particularly dopaminergic neuron are susceptible since dopamine in combination with oxygen & water results in the formation of dihydroxy phenyl acetic acid (DOPAC), ammonia and hydrogen peroxide (H₂O₂). This H₂O₂ further reacts with Fe²⁺ in dopaminergic neurons and forms hydroxyl radical (OH) and Fe³⁺ causing neurodegeneration (Chong et al., 2005).

6. **Microglial activation**: These are monocyte-activated immune-competent cells. They are activated by reactive oxygen species (ROS) system and damage neuron by producing cytokines.

Growing number of incidences from experimental and clinical studies strongly point out the active role of neuroinflammation in the etiopathogenesis of PD (Chen et al., 2008; Mosley et al., 2006; Chung et al., 2010)

Iron (Fe) is increasingly being implicated to play a role in the pathogenesis of PD. The physiological high iron content of the SN is even 35 % higher in PD with an elevation of Fe (III)/ Fe (II) - ratio to 1:2 (Berg et al., 2006). This means that the level of iron in 2+ form (reduced) is twice that of iron in the oxidized state in 3+ in PD, the former one which is far more deleterious.

While the etiology of the disease for most affected people remains unclear, mitochondrial dysfunction likely plays a key role in PD pathogenesis. Mitochondria are central not only to cell bioenergetics but also to apoptotic cell death (Shults, 2004). They are believed to play a fundamental role in ageing, and interact with specific proteins previously implicated in genetic forms of this neurodegenerative disease.
1.4. Implications of free radicals and associated oxidative/nitrosative stresses

Protective treatments have been proposed to suppress the possible causes of dopaminergic neurons apoptosis, such as-oxidative stress, age-dependent mitochondrial dysfunction, neurotoxins, decrease of neurotropic factors, excitotoxicity, disturbances of calcium homeostasis, immunologic and infectious mechanisms. (Naoi and Maruyama, 2001). Amongst these, oxidative stress has been suggested to play a major role.

ROS are an entire class of highly reactive molecules derived from the metabolism of oxygen. ROS, including superoxide radicals, hydroxyl radicals, and hydrogen peroxide, are often generated as byproducts of biological reactions or from exogenous factors. (Fig. 1.6)

ROS from both endogenous and exogenous sources may be involved in the etiologies of such diverse human diseases as arteriosclerosis, ischemic injury, cancer, and neurodegenerative diseases, as well as in processes like inflammation and ageing (Halliwell and Gutteridge, 1998; Gassen and Youdim, 1997).

![Figure 1.6: Schematic diagram of free radicals and endogenous antioxidants](image)

During pathological conditions and toxic insult, enhanced expression of COX-2 is seen leading to induction of free radicals that oxidizes dopamine, an important
neurotransmitter involved in the locomotion (Silvka and Cohen, 1985; Hastings, 1995).

The autooxidation of dopamine and subsequent polymerization to produce neuromelanin may form free radicals through the formation of toxic semiquinone derivatives or by the production of other potentially toxic species namely 6-hydroxy dopamine. The evidence available points to free radical mechanisms involving the altered mitochondrial function, and impairment in glutathione systems features, which may potentially be related to the mechanism of cell death in PD.

In this regard, different triggers, like oxidative damage to DNA, over activation of glutamate receptors, and disruption of cellular calcium homeostasis, different genetic and environmental factors, can activate a cascade of intracellular events that induce apoptosis (Nigel et al., 2004).

Further, it has been found, that nitric oxide (NO) is a highly fat-soluble free radical, having numerous promiscuous roles. NO synthesis is greatly amplified during inflammation. Several studies have demonstrated that inflammation correlates with the level of NO (Miller and Grisham, 1995).

However, rises in ROS and RNS, which may ultimately lead to neuronal cell death, do not necessarily reflect its primary cause, but can be a consequence of otherwise induced cellular dysfunction.

Excessive accumulations of iron, which are toxic to nerve cells, are also typically observed in conjunction with the protein inclusions. Iron and other transition metals such as copper bind to neuromelanin in the affected neurons of the SN (Fig.1.7). Neuromelanin may be acting as a protective agent.

**Figure 1.7: Neuromelanin granules in neurons**

The most likely mechanism is generation of reactive oxygen species (Jenner, 1998; Chiueh et al., 2000). Iron in black-staining granules of neuromelanin within neurons of the substantia nigra induces aggregation of synuclein by oxidative mechanisms (Kaur and Anderson, 2002). Similarly, dopamine and the byproducts of dopamine production enhance alpha-synuclein aggregation (Brain CAV annual meeting, Italy, 2010).
1.5. Role of Antioxidants in PD management

Hyperphysiological burden of free radicals causes imbalance in homeostatic phenomenon between oxidants and antioxidants in the body leading to oxidative stress. Researches in the recent past have accumulated enormous evidence advocating enrichment of body systems with antioxidants to correct vitiated homeostasis and prevent the onset as well as treat the disease fostered due to free radicals. Free radicals generated at high rates under pathophysiological conditions are insufficiently detoxified by endogenous scavengers.

Esposito et al suggest that there are many alternative antioxidant approaches that may be considered in future clinical trials including free radical scavengers, indigenous antioxidant enzyme boosters, iron chelators and drugs that interfere with the oxidative metabolism of DA in Parkinsonism (Tiwari, 2004).

The wide variety of approaches to rescue neurons includes free radical scavenging antioxidants, ion channel modulators, excitatory amino acid antagonists and neurotrophic factors. A number of dietary compounds with chemical antioxidant capacity are currently under investigation for their potential to protect against intracellular oxidative damage (Perez-Matube et al., 2012).

1.6. Role of Dopamine (DA) with dopamine and adenosine receptors

Dopamine (3,4-dihydrophenyl) ethylamine is an important brain chemical/catecholamine and when released into the synaptic cleft is broken down by monamine oxidase (MAO).

**Synthesis of DA**

Dopamine is principally synthesized from two key enzymes, dihydroxy phenylalanine (DOPA) and aromatic-L-amino acid carboxylase. Released dopamine acts on both receptors D1 and D2. The basal ganglia (BG), is thought to control movements by a delicate balance of transmission through direct and the indirect pathway. Direct pathway involves GABA as neurotransmitter. Stimulation of indirect pathway (inhibitory D2 receptors) inhibits the movement. In PD, there is loss of dopaminergic flow to the striatum and this has different effects on each pathway.

**In dopaminergic neurons:** L-tyrosine + THFA + O$_2$ + Fe$^{2+}$ $\rightarrow$ L- DOPA + DHFA + H$_2$O + Fe$^{3+}$ $\rightarrow$ Dopamine
The means by which cell damage occurs in PD is due to:-

L-tyrosine + Fe^{2+} + O_2 \rightleftharpoons L-tyrosine + Fe^{3+} + O_2^- \quad \text{(superoxide anion-most destructive)}

**In melanocytes:** L-tyrosine-----L-DOPA ----melanin (neuromelanin)

Healthy brains are supposed to be darker in the part of the brain called the substantia nigra because of neuromelanin.

Dopaminergic neurons are mainly found in three brain systems or pathways:-

- **a. Nigrostriatal system,** regulates the extrapyramidal system in the control of body movements
- **b. Mesolimbic system,** in the control of emotion and memory
- **c. And tuberoinfundibular system,** regulates the secretion of prolactin from pituitary gland which induces lactation in mammals

**Role of DA:**

1. Coordination of movement and locomotion
2. Feel good neurotransmitter and general arousal
3. Ability to learn and encoding of stimuli
4. Involved in focus, concentration, alertness
5. Libido effects
6. Learning and memory
7. Enhancement in verbal fluency and creativity
8. Decreased prolactin release
9. Sensoromotor responses

**Dopaminergic D2 (DAD2) receptors**

These are found in limbic areas, substantia nigra, striatum, nucleus acumbens and olfactory tubercle (Fig.1.8).

Dopamine receptors belong to the superfamily of G-protein coupled receptors characterized by attachment to a G-protein (guanine nucleotide -binding) protein, located on the inside of the receptor membrane. G-protein is a mediator to secondary messenger ATP, i.e. when

**Figure 1.8: Disrupted homeostasis levels of DA in Parkinson’s affected neurons**
receptor is activated; protein is negatively coupled to adenylate cyclase and inhibits ATP (Tortora and Grabowski, 1998)

**Adenosine A2 receptors**

Experimental findings have demonstrated the differential influence of adenosine A1 and A2 receptors on haloperidol-induced catalepsy and support the hypothesis that functional interaction between adenosine and dopamine mechanisms might occur through adenosine A2 receptors at the level of cholinergic neurons. Thus, A2 receptor antagonists may be of potential use in the treatment of PD (Mandhane et al., 1997).

**1.7. Therapeutic modalities**

The current therapeutic approach consists mainly on increasing the dopaminergic neurons activity or inhibiting the cholinergic effects to the striatum (Morais et al., 2003) (Table 1.1).

**Table 1.1. Currently used symptomatic treatment for PD**

<table>
<thead>
<tr>
<th>Class</th>
<th>Drugs</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>DA precursor</td>
<td>Levodopa (Stalevo)</td>
<td>Only 1 to 2% crosses blood brain barrier (BBB). Acts on both D1 and D2 receptors in striatum</td>
</tr>
<tr>
<td>Peripheral decarboxylase inhibitor</td>
<td>Carbidopa &amp; Benzserazide</td>
<td>Inhibits L- aromatic-amino-acid decarboxylase and increase t½ of levodopa and decrease the dose by ¼ of original.</td>
</tr>
<tr>
<td>Dopaminergic agonists</td>
<td>Bromocriptine, Pergolide, Piribidil, Ropinirole and Pramipixole</td>
<td>Bromocriptine is potent agonist on D2 but partial on D1. Pergolide is more potent than bromocriptine and acts on both D1 and D2 receptors. Piribidil-</td>
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<table>
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<tr>
<th><strong>MAO-B inhibitor</strong></th>
<th><strong>Selegeline</strong></th>
<th>This enzyme metabolizes dopamine. It is given with dopamine precursor at late stage of PD.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Anticholinergics</td>
<td>Procyclidin, Biperidin, Promethazine, Trihexylphenidyl, Orphinadine and</td>
<td>They basically act by balancing the unbalanced cholinergic activity in the striatum</td>
</tr>
<tr>
<td><strong>Dopamine Facilitators</strong></td>
<td><strong>Amantadine</strong></td>
<td>It is rapidly acting drug, which promotes presynaptic release of dopamine in brain.</td>
</tr>
<tr>
<td>Catechol-O-Methyl Transferase (COMT) Inhibitors:</td>
<td><strong>Tolcapone and Entacapone</strong></td>
<td>When peripheral metabolism of dopamine is blocked by carbidopa and benzserazide then it is metabolized by COMT. They are selective and potent COMT inhibitors</td>
</tr>
</tbody>
</table>

Some common side effects of AntiParkinson’s drugs:

- Postural hypotension
- Nausea vomiting
- Insomnia
- Confusion
- Gastrointestinal upset
- Increase in gonadotropin release
- Hallucination
- “Dyskinesia”

1.7.1. Alternative treatments

a. Fava and velvet beans are natural sources of L-DOPA and are taken by many patients. While they have shown some effectiveness (Katzenschlager et al., 2004; Raguthu et al., 2009) their intake is not free of risks
b. Surgery and deep brain stimulation

Deep brain stimulation (DBS) is presently the most used surgical mean of treatment but other surgical therapies consisting in producing lesions in specific subcortical areas are also effective (Nat Collaborating Center for chronic conditions, 2006). DBS involves the implantation of a medical device called a brain pacemaker, which sends electrical impulses to specific parts of the brain. Target areas for DBS or lesions include the thalamus, the globus pallidus (the lesion technique being called pallidotomy) or the subthalamic nucleus (Coffey, 2009).

c. Gene therapy

Gene therapy is currently under investigation (Obeso, 2010; Feng and Maguire-Zeiss, 2010). It involves the use of a non-infectious virus to shuttle a gene into a part of the brain. The gene used leads to the production of an enzyme, which, helps to manage PD symptoms or protects the brain from further damage. As of 2010, there are four clinical trials using gene therapy in PD.

d. Neural transplantation

Stem cells transplants have raised great recent interest. When transplanted into the brains of rodents and monkeys they survive and improve behavioral abnormalities (science daily). Nevertheless, while fetal stem cells are the easiest to manipulate their use is controversial.

1.7.2. Neuroprotective strategies

Diseases of the CNS particularly PD presents a challenge for the development of new pharmacological target. Current status of drug therapy provides only temporary relief from symptoms or they stop further deterioration rather than curing the disease. As a result, identification of new targets and targeting many of them simultaneously by offering neuroprotection with minimum or no side effect would be very beneficial to decrease or eliminate the disability associated with PD.

The concept of neuroprotection in therapeutic terms may be best described by (Shoulson., 1998) as "pharmacological interventions that produce enduring benefits by favorably influencing underlying etiology or pathogenesis and thereby forestalling
onset of disease or clinical decline." Our life span has increased and it brought about a significant increase in the incidence of neurodegenerative diseases.

Although these symptoms can be improved using currently available dopamine replacement strategies, there is still a need to improve current strategies of treating these symptoms, together with a need to alleviate non-motor symptoms of the disease. Moreover, treatments that provide neuroprotection and/or disease-modifying effects remain an urgent unmet clinical need (Meissner et al., 2011).

Mice when treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; 30 mg/kg i.p. twice, 16 h apart) and bromocriptine 10 mg/kg i.p stimulates antioxidant mechanisms in the brain and acts as a free radical scavenger in addition to its action at dopamine receptors, thus indicating its strength as a valuable neuroprotectant (Muralikrishnan and Mohanakumar, 1998).

Major findings have indicated in vitro free radical scavenging and antioxidant activities of ropinirole, a non-ergot DA agonist through activation of reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) mediated via DA D2 receptors as one of the mechanism of neuroprotection (Motoyuki et al., 1999).

The beneficial effects of selegiline (Deprenyl) in PD and other neurological disorders have generated excitement regarding exploration of other neuroprotective avenues (Ebadi et al., 2002).

The neuroprotective potential of non-steroidal anti-inflammatory agents (NSAID’s) against MPTP-mediated neurodegeneration (Teismann P and Ferger, 2001; Aguirre et al., 2008) has been revealed. The beneficial effects of COX-inhibitors in various neurodegenerative diseases (Dhir et al., 2007) have also been reported. The speculation of either COX-1 or COX-2 isoenzyme has neuroprotective potential in various models of neuronal injury (Teismann et al., 2003).

In addition, green and black tea extracts have shown to attenuate the neuronal apoptosis induced by 6-OHDA. This neuroprotection was attributed to the antioxidant actions of the polyphenolic constituents that have good capability to penetrate the BBB (Mandel et al., 2006).

Amongst the neuroprotective effects, the protective activity on the mitochondria by powerful antioxidant coenzyme Q 10 (Young et al., 2007), the polyphenolics from fruits (Joseph et al., 1999) and alpha lipoic acid (Palaniappan and
Dai, 2007), the anti-neuroinflammatory potential of omega-3-fatty acids docosahexaenoic acid (Mccann and Ames, 2005) have been revealed.

Agents currently under investigation as neuroprotective agents include anti-apoptotic drugs (TCH346, CEP-1347), antiglutamatergic agents, monoamine oxidase inhibitors (selegiline, rasagiline), promitochondrial drugs (coenzyme Q10, creatine), calcium channel blockers (isradipine) and growth factors (GDNF) (Obeso et al., 2010)

1.8 Biomarkers as indicators of disease process and therapeutic targets

A “biomarker” is generally defined as a measure of a biological process or other feature that can be correlated with some aspect of normal body function, an underlying disease process, or a response to a treatment. Biomarkers can be specific cells, molecules, or genes, gene products, enzymes, or hormones. Complex organ functions or general characteristic changes in biological structures can also serve as biomarkers (Fig.1.9).

Although the term biomarker is relatively new, biomarkers have been used in pre-clinical research and clinical diagnosis for a considerable time. For the clinician, there is hope that biomarkers will help diagnose symptomatic and presymptomatic disease or provide surrogate end-points to demonstrate clinical efficacy of new treatments, such as neuroprotective therapies (Michell et al., 2004)

While each neurodegenerative disease has its own characteristics and clinical manifestations, some common markers have been recognized. Among others, increased levels of oxidative/nitrosative damage to DNA, RNA, mitochondria, membranes, and proteins, etc. have been detected in connection with situations of neuronal damage.

The brain is made up of 70% lipid and any kind of stress is usually manifested by lipid peroxidative damage (Kedar, 2003).

In practical terms, this stress-induced lipid peroxidative damage in the brain can be quantified by either determining the amounts of per oxidative products or the rates of enzyme-catalysed reactions neutralizing free radical intermediates. Under normal conditions, decreased activity of antioxidant enzymes, viz. glutathione peroxidase (GPX) and catalase (CAT) in the brain leads to accumulation of oxidative free radicals resulting in degenerative effects (Naidu et al., 2003). Glutathione, a potent antioxidant plays an essential role in the dopamine turnover and pathogenesis
of PD (Slivka and Cohen, 1985; Hastings, 1995; Spina and Cohen, 1989). The estimation of the activity of such antioxidant enzymes such as superoxide dismutase (SOD) (EC 1.15.1.1), CAT (EC 1.11.1.6), GPx can be used to assess the therapeutic effects of different antioxidant agents.

Figure 1.9: Understanding the biomarkers involved in etiology and pathogenesis of PD (Sporadic)

Protein carbonyls formation has been indicated to be an earlier marker of protein oxidation for the oxidant/antioxidant barrier impairment in various inflammatory diseases (Rajesh et al., 2004).

Malondialdehyde: total antioxidant capacity (MDA: TAC) ratio as novel indicator of oxidative stress in aging to monitor and optimize antioxidant therapy which may reduce morbidity and perhaps increase the healthy, useful life span of an individual (Suresh et al., 2010).

Lipid per oxidation (LPO), oxidative hemolysis and plasma ceruloplasmin (CPO) are significantly higher in PD patients as compared to normals (Rao et al., 2003).
Recent evidence suggests that CPO, a copper-containing enzyme exhibits potent pro-oxidant activity and causes oxidative modification of important biomolecules like low density lipoproteins (Sontakke and More., 2004)

In clinical studies where analysis of antioxidant status is important, oxygen radical absorbance capacity (ORAC) method has been used to evaluate the hydrophilic and lipophilic antioxidants in postmortem brain tissue.

Activation of microglia following neuroinflammation up-regulates nitric oxide synthase (NOS) and COX expression (Teismann and Ferger, 2001; Mosley et al., 2006; Watanabe et al., 2008). Nitric oxide (NO) is one of free radicals and cell signaling molecule. It was reported that NO, as a toxic factor, could mediate the death of dopaminergic neurons (Lavoie and Hastings, 1999). Peroxynitrite- and nitrite-induced 1-Methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) that is accumulated in astrocyte is converted to its toxic metabolite 1-methyl-4-phenylpyridinium ion (MPP+), by the enzyme monoamine oxidase-B (MAO-B), which is localized predominantly in astrocytes were shown to have a high capacity for MPP+ retention and efflux (Di Monte et al., 1992).

Myeloperoxidase (MPO) activity, an important marker of tissue damage involving inflammatory cells caused by disease or environmental toxins (Gupta et al., 2009).

Increased brain metal levels are associated with normal aging and a variety of degenerative diseases. Animal studies and post mortem analyses of human brain have revealed increased iron levels in substantia nigra as well as reduced neuromelanin content. High levels of copper have shown pro-oxidant activity (Sedighi et al., 2006).

The resulting increase in the extracellular glutamate levels causes secondary excitotoxic damage to surrounding cells via chronic glutamate receptor overstimulation (Babu and Bawari, 1997; Didier et al., 1996).

The concentrations of dopamine (DA) and norepinephrine (NE) which gets depleted in PD can be determined by sensitive trihydroxyindole fluorometry for the simultaneous estimation of norepinephrine and dopamine in tissue (A. F. Hogans. personal communication)

Mitochondrial complex I dysfunction is implicated in the pathogenesis of neurodegenerative disorders such as Parkinson’s disease. Complex I dysfunction has been identified in mitochondria from platelet, brain, and muscle of Parkinson’s
disease patients (Parker et al., 1989; Mizuno et al., 1995). Identification of factors involved in maintenance and restoration of complex I function could potentially help to develop prophylactic and therapeutic strategies for treatment of this class of disorders (Kenchappa and Ravindranath, 2003).

The traditional histopathology and sensitive degeneration stains represents an inexpensive route to obtain the needed advances in neurotoxicity assessment with respect to added sensitivity and specificity (O’Callaghan and Sriram, 2005). Thus, whereas histological features and biochemical changes associated with specific neurological disease states can be identified in postmortem brain tissue from humans, or from brain tissue prepared from animal models of a given disease condition. So, broadly applicable biochemical markers for detecting all types of neurotoxic effects and behavioural test methods are currently used as a ‘rodent neurological exam.’

It is unlikely that a single marker will provide sufficient sensitivity and specificity for early diagnosis or prognosis. A combination of different biomarkers will be vital for the development of objective end-points for future neuroprotection trials. Given their accessibility, the development of blood biomarkers would be a major advancement (Meissner et al., 2011).