Metal Exposure and Oxidative Stress Induced Parkinson’s Disease

Authors
Adnan Bashir Bhatti, MD¹, Muhammad Usman, MBBS²

¹Department of Medicine, Capital Development Authority (CDA) Hospital, Islamabad, Pakistan
Email: dr.adnanbashir@gmail.com
²Department of Medicine, Jinnah Hospital Lahore (JHL), Pakistan
Corresponding Author
Adnan Bashir Bhatti, MD
Email: dr.adnanbashir@gmail.com

Abstract
Parkinson’s disease (PD) is a neurodegenerative disorder characterized by dopamine deficiency and loss of neurons in the Substantia Nigra (SN) of brain. The deficiency of dopamine, thereafter, presents in the form of a number of motor and non-motor deficiencies and complications. Our understanding about PD is still evolving and treatments available for this disease are still quite basic. Therefore, better understanding of the pathological events linked with this disease may help us devise better treatment protocols of the disease.

Increased production of reactive oxygen species (ROS), oxidative stress, seems to be the centerpiece of PD pathology. Increased oxidative stress in PD is mainly the result of by-products generated from dopamine metabolism, mitochondrial dysfunction, and inadequate activity of the brain’s antioxidant system. Once generated, ROS cause damage to the brain due to its high unsaturated fatty acid content, which makes it quite sensitive to oxidative injury.

In our daily life we’re exposed to different metals and metals have been long linked as risk factors for PD development. Therefore, in this article we’ve considered three important metals (iron, aluminum, and copper), most of which we encounter time to time in domestic, occupational and environmental standpoint. This review, therefore, is concerned with discussing the role of these metals with increased oxidative stress in brain. Moreover, this review will also discuss how this increase in oxidative stress is linked to various component of PD pathology.

Introduction
The history of Parkinson’s disease (PD) dates back to two centuries from now when James Parkinson wrote “An essay on the shaking palsy”, in 1817 (¹). More than 100 years later, dropout of cells in the substantianigra (SN) of brain was pointed out to be the key lesion in PD and 140 years after the initial mention of PD, dopamine was discovered as a putative neurotransmitter involved in the pathogenesis of this disease (²). Discovery of
dopamine deficiency and success of levodopa treatment in improving PD symptoms was heralded as a huge success in PD treatment (3) (4) (5) (6). But even today, our understanding about PD is still incomplete and the treatment options available are only symptomatic. Till date, there is no neuro-protective or neuro-regenerative intervention that has proved to be effective in treating PD (7) (8). Therefore, knowing more about the risk factors that increase neuronal breakdown in PD may improve our understanding of the disease and may help us come up with better treatment modalities.

**Motor and Non-Motor Manifestations in PD**

PD is a chronic neurodegenerative disorder with an array of motor and non-motor symptoms, which are believed to be the result of dopaminergic neuronal loss (8). The motor manifestations in PD include bradykinesia (9); rest tremors (10); rigidity with postural deformities, postural instability, motor blocks (freezing) (11); re-emergence of primitive reflexes (12) (13); orofacial-larygenal rigidity and bradykinesia leading to sialorrhoea, dysarthria, dysphagia and hypophonia (10) (14); neuro-ophthalmological deficits including decreased rate of eyelids blinking, spasm of convergence, reduced tear film, exposure keratitis, blephrospasm and impaired vision (15); and respiratory insufficiency (16). Non-motor manifestations of PD include autonomic dysfunction with orthostatic hypotension, dysfunction of sweating, erectile dysfunction and sphincter paralysis (17) (18) (19); cognitive decline (20); neurobehavioral abnormalities like apathy, depression, anxiety, and even hallucinations (21); sleep disorders (22) and sensory abnormalities like pain (23). There has been much clarity in our understanding of underlying causes of nigral cell damage. Among the mechanism involved, genetic mutations (7), mitochondrial dysfunction (24), denaturation and aggregation of proteins (25), inflammation (26) (27), and oxidative stress (29) seem to be the most important mechanisms involved in PD pathogenesis.

**Role of Oxidative Stress in PD Pathology**

Human brain consumes as much as 20% of body oxygen and a lot of this oxygen is converted into reactive oxygen species (ROS) (28). Both neuronal and glial cells are responsible for the generation of ROS inside the brain and disequilibrium in the electron transport chain is the main contributor of oxidative stress at the cellular level (29). As for oxidative stress in PD, both increased production and impaired clearance of ROS seem to be the culprit behind increase in oxidative stress. The prime factors contributing to increase ROS include dopamine metabolism and mitochondrial dysfunction. Whereas, reduced clearance of ROS is mainly due to the decrease in the concentration of catalase and glutathione peroxidase (30).

**Role of Dopamine**

Dopamine is an unstable neurotransmitter that undergoes auto-oxidation- a reaction catalyzed by metals, enzymes like tyrosinase and oxygen- to form dopamine quinones and free radicals (31). Under normal circumstances, monoamine oxidase A (MAO-A), which is largely localized within
catecholaminergic neurons, metabolizes and regulates the levels of dopamine \(^{(32)}\). However, with age or neurodegenerative disease like PD, the level of MAO-B increases within glial cells and this MAO-B becomes the key enzyme metabolizing dopamine \(^{(33)}\) \(^{(34)}\). The products of MAO-B metabolism include an ammonia derivate (3, 4-dihydroxyphenyl-acetaldehyde) and hydrogen peroxide (H2O2). H2O2, being highly permeable, enters the neighboring dopaminergic neurons and produces ROS like hydroxyl radicals after reacting with cellular Ferrous ions (Fe+2) \(^{(35)}\) \(^{(36)}\). In addition, dopamine quinones can exacerbate neuro-degeneration too \(^{(37)}\). The main mechanism through which dopamine quinones trigger oxidative stress is their cyclization to aminochrome, which is a reactive molecules that depletes cellular NADPH and initiates the production of superoxide ions \(^{(38)}\). Finally, the concentration of free dopamine in neuronal cytoplasm can also trigger oxidative stress. Vesicular monoamine transporter 2 (VMAT2) and dopamine transporters (DAT are concerned with the sequestration of cellular dopamine within storage vesicles and reuptake of dopamine from synaptic clefts respectively. Perturbations in the functioning of these transporters can increase the concentration of free dopamine within cell cytoplasm and can cause a resultant increase in oxidative stress \(^{(39)}\) \(^{(40)}\).

**Role of Mitochondrial Dysfunction**

Mitochondrial is a dynamic organelle and controls several vital functions. As mentioned before, it has been repeatedly reported that dysfunction of electron transport chain is the main contributor of oxidative stress within cells. Several components and processes of electron transport chain like complex I, II, III, dehydrogenases in the tricarboxylic acid (TCA) and final coupling of oxygen with hydrogen ions to form water result in the generation of superoxide anions \(^{(41)}\) \(^{(42)}\) \(^{(43)}\). These anions are then turned into H2O2 by the action of manganese superoxide dismutase (MnSOD) \(^{(44)}\). The role of mitochondria in causing oxidative stress was first elucidated when Parkinsonism was observed in some 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) drug abusers \(^{(45)}\). MPTP is taken by brain astrocytes where it is converted into 1-methyl-4-phenylpyridinium (MPP+) by the action of MAO-B. MPP+ is a known inhibitor of complex I of electron transport chain. This way it inflicts damage to NS dopamine neurons by causing a drop in the level of ATP, increased oxidation of dopamine and enhanced production of ROS \(^{(46)}\).

**Role of Anti-Oxidant System**

As mentioned former in the text, increased oxidative stress in PD is the result of both increased production and decreased clearance of ROS. Analysis of serum superoxide dismutase (SOD) and glutathione peroxidase (GSH) activity in controls and patients with PD showed a marked elevation in the activity of these anti-oxidant enzymes, which further proves the notion that increased oxidative stress is the centerpiece of PD pathology \(^{(47)}\). Moreover, postmortem analysis of brain tissues in patients with PD showed marked reduction in the level of GSHin the SN of PD.
patients when compared to the control (48) (49). The level of catalase was found to have undergone significant reduction in patients with PD (50). Similarly, decreased level of other antioxidant like GSH peroxidase and ceruloplasmin are also observed in patients with PD (51) (52).

How ROS in PD Damage the Brain?
Human brain contains abundant polyunsaturated acids like arachidonic acid and docosahexaenoic acid. These acids are unsaturated and are highly susceptible to peroxidation induced by ROS. In fact, this is the basic mechanism of ROS induced brain injury (53). Structural damage to the membranes caused by lipid peroxidation and exaggerated injury induced by lipid oxidation production are the further predictors of brain injury (54) (55). One such product of lipid peroxidation is 4-Hydroxyl-2-nonenal (HNE). HNE is a highly reactive molecule that can inflict damage to the cells by denaturing proteins, causing a decrease in GSH levels, DNA fragmentation and apoptosis triggered by the activation of capsases (56) (57). The notion that lipid peroxidation and damage inflicted by by-products of lipid peroxidation might contribute to PD progression is supported by several clinical studies as well. The results of several studies have backed the fat the level of brain unsaturated fatty acids decrease, while the level of lipid peroxidation products like HNE and malondialdehyde increase in the brain substance and cerebrospinal fluid of PD patients (58) (59) (60).

Since urbanization and industrialization, our day to day exposure to metals has increased significantly. We are subjected to metals of different sorts daily via domestic routes, industries and environmental exposure. Metal exposure is a known risk factor for PD. Therefore, the purpose of this review is to find a link between exposure to metals and increased risk of PD. Moreover, this review will also shed some light on the underlying mechanism of metal induced oxidative stress, which maybe the main event responsible for metal induced PD.

Discussion
Exposure to metals is an important risk factor for different neurological disorders. The exposure to metals can be domestic or occupational. Different metals are recognized to cause increased risk of neurological disorder by increasing the production of ROS (61). In this review, we've discussed different metals (Iron, Aluminum and Copper) and their role in the pathogenesis of PD (Table 1). These metals are of particular interest and are discussed here because a lot of household appliance, utensils (especially cooking utensils) make use of these metals.

Iron
Among metals that are linked with PD, iron is the most important. Postmortem studies of the brain tissues in patients with PD showed significant increase in the iron content of SN (63) (64) (65). In the normal brain tissues, iron is extensively distributed within different areas of basal ganglia including globus pallidus, SN and putamen (66). However, this pattern of iron distribution changes significantly with increasing age and
neurodegenerative disease like PD. In such conditions, the levels of iron become abnormally high in SN, when compared to other areas of basal ganglia\(^{67}\).

Iron seems to influence PD, both by increasing oxidative stress and decreasing anti-oxidant enzymes. Ferrous (Fe+2) and ferric (Fe+3) forms of iron can trigger a chain reaction after interacting with superoxide and hydrogen peroxide (Fenton’s reaction) respectively. This chain reaction results in the formation of highly reactive hydroxyl radicals, which can trigger neurotoxicity \(^{(68)}\) \(^{(69)}\). In addition, iron can increase the level of oxidative stress by causing mitochondrial dysfunction. Iron seems to increase the production of ROS by blocking the activity of complex I and complex IV of electron transport chain\(^{(70)}\). Moreover, excess of iron has been found to decrease the level of antioxidant enzymes—which is perhaps due to increased oxidative stress especially glutathione \(^{(71)}\). Iron content and increased oxidative stress are two way process i.e. higher iron content can induce increased oxidative stress and higher oxidative stress can in turn increase iron content too. Iron is release from ferritin by the action of superoxide anions, from iron-sulfur proteins via the action of peroxynitritite and from heme compounds by the action of peroxidase \(^{(72)}\) \(^{(73)}\) \(^{(74)}\). Exposure to excess iron via different routes (oral or parenteral) can increase the risk of PD as well. Kaur et al. 2007, conducted an experiment on mice, the results of which showed that increased oral intake of iron during early stages of life made the mice more susceptible to develop PD later in their life. Such mice developed symptoms of dopamine deficiency by the time they reached 12 months and showed significant loss of nigral neurons by the time they were 24 months old. Moreover, the brain of such mice showed increased vulnerability to oxidative stress induced injury \(^{(75)}\). Similar results were obtained when iron was given orally or was injected within SN of mice. The higher level of iron in SN resulted in reduced levels of GSH and increased levels of hydroxyl radicals \(^{(76)}\). When iron chelators, like desferrioxamine, are given intramuscularly, they result in significant reduction in brain iron level and exert a neuro-protective effect against MPTP and iron in mice models \(^{(77)}\). All these evidences cumulatively suggest that increased iron in the SN is an important trigger for oxidative stress and resultant neuro-degeneration in PD.

**Aluminum**

Aluminum is a non-redox metal that is well known to have profound relation in the pathogenesis of several neurodegenerative diseases \(^{(78)}\) \(^{(79)}\). The X-ray microanalysis of brains of patients with PD showed increased level of two metals in SN: one metal being iron and other being Aluminum \(^{(80)}\). Although, aluminum is a non-redox metal, it is believed to contribute in the pathogenesis of PD and other neurodegenerative disorder through multiple mechanisms \(^{(81)}\). The main mechanism through which aluminum seems to increase oxidative stress is by increasing iron induced oxidative stress. Aluminum brings alterations in membrane structure, which increases the
susceptible of membrane to undergo iron induced peroxidation \(^{(82)}\). However, aluminum can induce membrane oxidation through a mechanism independent of iron too \(^{(83)}\). Another mechanism through aluminum contributes to increased oxidative stress in brain, independent of iron, is through the increased oxidation of NADPH \(^{(84)}\). Not only that, but aluminum also contributes to oxidative stress in PD by increasing the production of hydroxyl oxygen species through a mechanism independent of iron \(^{(85)}\). In fact, in aluminum induced brain injury, nitric oxide (NO) comes into play, which interacts with superoxide anions to form peroxynitrite anions that are even more toxic than simple hydroxyl anions \(^{(86)}\). Like iron, another mechanism through which aluminum might induce oxidative stress mediated damage in through mitochondrial dysfunction. This maybe the result of dysfunction of mitochondrial enzymes like cytochrome c oxidase (COX) and Complex I \(^{(87)}\). Not only that, aluminum seems to increase oxidative stress by decreasing the level of different antioxidants as well. Anuradha et al. 2014, demonstrated that sub-acute exposure of rats to aluminum causes significant reduction in the level of GSH and activity of SOD, CAT, and glutathione peroxidase (GPx) \(^{(88)}\).

Moreover, occupational exposure to aluminum is a risk factor for PD \(^{(89)}\). In fact, there have been some case reports were exposure to aluminum compounds was linked with disequilibrium of dopaminergic system \(^{(90)}\). Furthermore, results obtained from animal models have suggested that intra-peritoneal injections of aluminum chloride can increase oxidative stress and resultant neuronal loss in areas of brain stem \(^{(91)}\). All these evidence suggest that both oral and parenteral exposure to aluminum maybe a risk factor for increased oxidative stress and disequilibrium of dopaminergic system of brain.

**Copper**

Copper is another metal that has been linked with PD. The link of copper with PD is particularly interesting. Unlike iron and aluminum, the concentration of copper in SN decreases significantly. It has been repeatedly demonstrated that patients with PD always have higher iron in their SN and lower level of copper \(^{(92)}\) \(^{(93)}\). This apparent paradox can be explained on the basis of copper-dependent ferroxidase system activity. Ferroxidase promotes the conversion of Fe+2 into Fe+3, so that it can be moved out of the cells. The activity of ferroxidase is controlled by copper \(^{(94)}\). But, the activity of ferroxidase is found to diminish greatly both within the plasma and cerebrospinal fluid (CSF) of patients with PD \(^{(95)}\) \(^{(96)}\) \(^{(97)}\). Therefore, it is quite possible that decreased concentration of copper in SN is the consequence of defective function of ferroxidase \(^{(98)}\).

As for copper induced oxidative stress, it seems to be the result of a mechanism similar to iron. Copper catalyzes the conversion of hydrogen peroxide into hydroxyl group through this reaction \((\text{Cu}^+ + \text{H}_2\text{O}_2 \leftrightarrow \text{Cu}^{++} + \text{OH}^- + \text{OH})\) \(^{(99)}\). Alpha-Synuclein is a protein of unknown significance that is found in higher concentrations in the form of Lewy bodies within the brains of PD patients \(^{(100)}\). These proteins bind with copper to enhance
their redox capacity. After that, this complex produces H2O2 from ascorbic acid. This H2O2 then triggers the oxidation of dopamine and production of more neurotoxic metabolites \((101)\). As for lipid peroxidation, copper causes oxidative stress mediated damage to membranes through both iron dependent and independent mechanisms \((102)\) \((103)\). Although copper is an essential cofactor for several mitochondrial enzymes, but its overload may cause mitochondrial dysfunction too. Copper overload has been linked with complex IV (cytochrome C oxidase, COX complex) dysfunction \((104)\). The Cu-Alpha-Synuclein complex also seems to decrease the level of antioxidants like GSH \((101)\). In addition, the malfunction ceruloplasmin (a potent antioxidant) has been reported in patients with PD \((105)\). Several studies have declared copper to as a risk factor for PD \((106)\) \((107)\). On the other hand, there are some studies were exposure to copper and risk for PD was barely of statistical significance \((108)\). More research may be needed in this area.

**Table 1:** Summary of metal exposure and PD pathology

<table>
<thead>
<tr>
<th>Metal</th>
<th>Brain levels (SN)</th>
<th>Major reaction for ROS generation</th>
<th>Mitochondrial dysfunction</th>
<th>Lipid peroxidation</th>
<th>Decrease/malfunction in Antioxidant activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td>Increased</td>
<td>Fenton’s reaction</td>
<td>Complex I and IV</td>
<td>Independent mechanism</td>
<td>GSH</td>
</tr>
<tr>
<td>Aluminum</td>
<td>Increased</td>
<td>Iron induced and peroxynitrites</td>
<td>Complex I and IV</td>
<td>Iron induced and independent mechanism</td>
<td>GSH, SOD, CAT, GPx</td>
</tr>
<tr>
<td>Copper</td>
<td>Decreased</td>
<td>Fenton’s reaction</td>
<td>Complex IV</td>
<td>Iron induced and independent mechanism</td>
<td>Ceruloplasmin, GSH</td>
</tr>
</tbody>
</table>

GSH: Glutathione, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase.

**Conclusion**

From this review it can be safely concluded that increased exposure to the aforementioned metals could be a marker or at least an important risk factor for the development of PD. Increase in the oxidative stress is perhaps the key mechanisms through which these metals disturb SN dopaminergic neurons and their functioning in PD. Therefore, interventions that could specifically target different mechanisms that reduce oxidative stress can be an effective tool in the fight against PD, which are still largely treated symptomatically. Moreover, devising the cut off limits to the exposure to the aforementioned metals may provide valuable results too. However, it must also be kept in mind here is whether domestic exposure to these metals poses similar threat of developing PD as occupational or experimental exposure do is still a matter of debate. More research is needed in this area.
References


36. Hastings TG. The role of dopamine oxidation in Mitochondrial dysfunction:


67. Sian-Hulsmann J, Mandel S, Youdim MB, Riederer P. The relevance of iron in the


83. Verstraeten SV, Oteiza PI. Effects of Al(3+) and related metals on membrane phase state and hydration: correlation with


89. Gorell JM, Rybicki BA, Cole Johnson C, Peterson EL. Occupational exposure to specific metals (manganese, copper, lead, iron, mercury, zinc, aluminum and others) appears to be a risk factor for Parkinson’s disease (PD) in some, but not all, case-control studies. Neuroepidemiology. 1999;18:303–8.


96. Boll MC, Alcaraz-Zubeldia M, Montes S, Rios C. Free copper, ferroxidase and SOD1 activities, lipid peroxidation and NOx content in the CSF. A different marker profile in four neurodegenerative


