Oxidant-antioxidant system: Role and significance in human body

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Present article gives a holistic view of the causes, role and control of oxidative stress in the development and progression of various human diseases. Several types of reactive species are generated in the body as a result of metabolic reactions in the form of free radicals or non-radicals. These species may be either oxygen derived or nitrogen derived and called pro-oxidants. They attack macromolecules including protein, DNA and lipid etc., causing cellular / tissue damage. To counter their effect, the body is endowed with another category of compounds called antioxidants. These antioxidants are produced either endogenously or received from exogenous sources and include enzymes like superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, minerals like Se, Mn, Cu and Zn, and vitamins like vitamin A, C and E. Other compounds with antioxidant activity include flavonoids, bilirubin and uric acid etc. In a healthy body, pro-oxidants and antioxidants maintain a ratio and a shift in this ratio towards prooxidants gives rise to oxidative stress. This oxidative stress may be either mild or severe depending on the extent of shift and remains the cause of several diseases such as cardiovascular diseases, neurological diseases, malignancies, renal diseases, diabetes, inflammatory problems, skin diseases, aging, respiratory diseases, liver diseases and different types of viral infections. As more and more reports are pouring in, a lot of information is being unfolded about oxidative stress in relation to several other diseases.

Chemical compounds and reactions capable of generating potential toxic oxygen species/free radicals are referred to as pro-oxidants. On the other hand, compounds and reactions disposing off these species, scavenging them, suppressing their formation or opposing their actions are called anti-oxidants. In a normal cell, there is an appropriate prooxidant: antioxidant balance. However, this balance can be shifted towards the prooxidant when production of oxygen species is increased or when levels of anti-oxidants are diminished. This state is called oxidative stress and can result in serious cell damage if the stress is massive or prolonged. Oxidative stress is implicated in the etiopathogenesis of a variety of human diseases.1-4

During the last two decades, there has been a growing interest in studies that concern with the prevention of uncontrolled oxidative process leading to various diseases in living system. Several studies have shown the role of oxidative stress in the causation and progression of different diseases including atherosclerosis, carcinogenesis, neurodegenerative diseases, chronic inflammatory diseases, radiation damage, aging and various other pathobiological effects.5-6 This definitely developed a responsibility to scientists, medical practitioners and clinical epidemiologists to find out the exact role and the control of oxidant-antioxidant system in human diseases.

In view of an enormous data from several studies showing a clear implication of oxidative stress in causation and progression of various diseases globally, it appeared worth to have an updated knowledge in this area. Present review presents a holistic picture of various findings on the role and significance of oxidant-antioxidant system in various important diseases.

The oxidants/free radicals are species with very short half life, high reactivity and damaging activity towards macromolecules like proteins, DNA and lipids. These species may be either oxygen derived (ROS, reactive oxygen species) or nitrogen derived (RNS, reactive nitrogen species). The oxygen derived species include O2• (superoxide), HO (Hydroxyl), HO2 (Hydroperoxyl), ROO• (Peroxyl), RO (Alkoxyl) as free radicals and H2O2 (Hydrogen peroxide), HOCL (Hyochlorous acid), O3 (Ozone), and 'O2 (Singlet oxygen) as non-radicals. Similarly, nitrogen derived oxidant species are mainly NO (Nitric oxide), ONOO• ( Peroxynitrite), NO2 (Nitrogen dioxide) and N2O5 (Dinitrogen trioxide).7

Reactive Oxygen Species

The exogenous sources of ROS include electromagnetic radiation, cosmic radiation, cigarette smoke, car exhaust, UV light, ozone (O3), and low wave length electromagnetic radiations. Similarly, the endogenous sources of ROS are mitochondrial electron
transport chain, respiratory burst by phagocytes, beta oxidation of fat in peroxisome, auto-oxidation of amino acids, catecholamines, haemoglobin and ischaemia reperfusion injury. Superoxide anion radical \((O_2^-)\) regulates metabolites capable of signalling and communicating important informations to the cellular genetic machinery. Over production of \(O_2^-\) takes place in various chronic inflammatory cases, induced by drug, toxin, stress, tissue injury and heavy exercises. Hydroxyl radical is another damaging radical with a half life of \(10^{-5}\) sec and produced from \(H_2O_2\) and \(O_2^-\) by Haber-Weiss Reaction. Some HO may be produced from hypochlorous acid in phagocytic cells. Similarly, \(H_2O_2\) is a relatively stable, poorly reactive, non-radical oxygen species which easily crosses cell membrane and attacks different sites by converting into HO. This is produced by dismutation of \(O_2^-\) by superoxide dismutase (SOD) and finally meets many fates including its reduction to water. \(H_2O_2\) is also involved in the generation of free radicals in presence of transitional metal ions.

Of the reactive nitrogen species (RNS), nitric oxide is the most important nitrogen derived physiologically free radical and one of the 10 smallest molecules in nature with molecular weight of 30 Da. It was once considered as a mere toxic pollutant. However, later studies reported it to relax smooth muscle and involved either in the causation or recovery of several diseases. NO is synthesised from L-arginine by a family of enzymes, NOS (Nitric oxide synthase), in a two step process.

**Reactive Nitrogen Species**

NO rapidly undergoes addition, substitution, redox and chain terminating reactions. These reactions serve as the molecular basis for its biological effects in human body. The target molecules of NO are intracellular thiol and metal containing proteins and low molecular weight thiols like glutathione and cysteine etc. NO acts as a “Double edged Sword” in health and disease. The main physiological role of NO is controlled by Type I and III NOS expression via intracellular Ca-calmodulin complex dependant mechanism. Both deficiency and excess of NO are believed to be involved in different pathophysiological states like stroke of brain, ischaemia, gastrointestinal dysfunctions, achalasia, Hirschsprung’s disease, congenital hypertrophic pyloric stenosis etc.

Increased type II NOS (iNOS) expression and NO production occur in bacterial sepsis (septic shock), eclampsia and hyperdynamic states like laenne’s cirrhosis. This also plays a role in inflammatory diseases like bronchial asthma, arthritis and ulcerative colitis via different cytokines and Th2 cell stimulation. In diabetes mellitus, inducible NOS (iNOS) over expression plays a role in β cell destruction by the inhibition of mitochondrial enzymes and via different cytokines release from activated lymphocytes.

Peroxynitrite (ONOO⁻) is another powerful oxidant that interacts with a wide range of targets to cause tyrosine nitration, thiol oxidation, lipid peroxidation, DNA strand break, guanosine nitration/oxidation and ultimately cell death. The reaction of ONOO⁻ with excess NO generates \(N_2O_3\), which can combine with more NO to form \(N_2O_5\) to cause nitrosative stress.

When overall generation of ROS and RNS exceeds the total antioxidant activity in the body, the resulting condition is called oxidative stress. This stress may be mild or severe. Based on various reports, the causes and after effects of mild and severe oxidative stresses are shown in the flow chart.

**Preventive Antioxidants, First Line Defense**

To counter the harmful effects of ROS and RNS, antioxidant defense mechanism operates to detoxify or scavenge these reactive oxygen species. The antioxidant system comprises different types of functional components classified as first line, second line, third line and fourth line defenses. The first line defense comprises preventive antioxidants that act by quenching of \(O_2^-\), decomposition of \(H_2O_2\) and sequestration of metal ions. The antioxidants belonging to this category are enzymes, like superoxide dismutase (SOD), catalase, glutathione peroxidase, and glutathione reductase and non-enzymatic molecules like minerals and some proteins. Superoxide dismutase mainly act by quenching of superoxide (\(O_2^-\)), an active oxygen radical, produced in different aerobic metabolism. Catalase is a tetrameric enzyme, present in most of the cells and acts by catalysing the decomposition of \(H_2O_2\) to water and oxygen. Glutathione peroxidase (GPx) is a selenium containing enzyme which catalyses the reduction of \(H_2O_2\) and lipid hydroperoxide (LO₂H), generated during lipid peroxidation, to water using reduced glutathione as substrate.

The antioxidant minerals include Se, Mn, Cu, and Zn and function primarily in the metalloenzymes. Se is required in some of the immune mechanisms and biosynthesis of ubiquinone and ATP in mitochondria. The renal cortex, pancreas, pituitary and liver contain high amounts of selenium. A deficiency of selenium produces hepatic necrosis, muscular dystrophy, necrosis...
of cardiac muscle and other disorders in various experimental animals. Selenium and vitamin E both appear to be necessary for efficient scavenging of peroxides from cytosol and cell membrane, respectively. Mn exerts its antioxidant action through mitochondrial SOD (MnSOD) which catalyses the dismutation of oxygen radical (O$_2^-$) produced during aerobic metabolism in mitochondria. Cu is present in a number of important metalloenzymes including cytosolic SOD, cytochrome oxidase, dopamine β-hydroxylase, lycop oxidase and ascorbic acid oxidase etc. This exerts its antioxidant activity through the cytosolic superoxide dismutase. Zinc is an element essential for normal growth, reproduction and other different functions of the body. It is a component of several enzymes like cytosolic superoxide dismutase, alcohol dehydrogenase, alkaline phosphatase, carbonic anhydrase etc.

**Radical Scavenging Antioxidants, Second Line Defense**

The antioxidants belonging to second line defense include glutathione (GSH), vitamin C, uric acid, albumin, bilirubin, vitamin E (mainly α-tocopherol), carotenoids, flavonoid and ubiquinol. Glutathione (GSH, gamma glutamyl cysteinylglycine) is the most abundant non-protein thiol, synthesised in the liver and acts as a substrate for glutathione peroxidase enzyme. This also serves as a scavenger of different free radicals. Similarly, β-carotene (Pro-vitamin A), vitamin C and vitamin E are some important scavenging antioxidant vitamins which cannot be synthesized by most mammals including human beings and therefore, are required from diet.

**Responses and signals during oxidative stress**

<table>
<thead>
<tr>
<th>Mild oxidative stress (positive effect)</th>
<th>Severe oxidative stress (negative effect)</th>
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<td>Physiological signal for beneficial cellular response</td>
<td>Excess ROS/RNS &amp; Low antioxidant defense</td>
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<tr>
<td>Activation of signal transduction mechanism</td>
<td>Damage to biomolecules (Lipid, DNA, Protein)</td>
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<tr>
<td>Cell differentiation</td>
<td>Lipid peroxidation (Damage to membrane ion channel, ion transporters)</td>
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<tr>
<td>Apoptosis</td>
<td>DNA damage (Strand breakage of Base, Modification)</td>
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<td>DNA repair</td>
<td>Protein damage (Damage to receptor, enzyme, ion channel)</td>
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<tr>
<td>Cell cycle arrest</td>
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<tr>
<td>Induction of antioxidants</td>
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<tr>
<td>Tissue protection</td>
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The retinoids like Beta-carotene, provitamin A and Vitamin A, are essential for vision, reproduction, growth and maintenance of epithelial tissues.β-carotene is an established and excellent scavenger of singlet oxygen, produced during photosensitivity. Vitamin C and dehydroascorbic acid interact directly with radicals like O₂⁻ and HO in plasma, thus preventing damage to red cell membrane. It probably assists α-tocopherol in inhibition of lipid peroxidation by recycling the tocopherol radical. Ascorbate (radical) is reduced by intracellular GSH. It is a good scavenger of many free radicals like O₂⁻, HO and various lipid hydroperoxides and may help to detoxify many inhaled oxidizing air pollutants like ozone and NO₂ and free radicals in cigarette smoke in the respiratory tract.

Vitamin E comprises a group of eight naturally occurring related tocopherols, most important being α-tocopherol. This one is the most important and least toxic of all lipid soluble anti-oxidant vitamins. It scavenges peroxyl radical intermediates in lipid peroxidation and is responsible for protecting PUFA (poly unsaturated fatty acid) present in cell membrane and low density lipoprotein (LDL), against lipid peroxidation.

Flavonoids are phenolic compounds, present in several plants, which inhibit lipid peroxidation and lipoxygenases in vitro and in presence of free metal ion (Fe³⁺). They act as pro-oxidant. Bilirubin and uric acid also act as antioxidants.

Repair and De-Novo Enzymes, Third Line Defense

Third line antioxidants are a complex group of enzymes for repair of damaged DNA damaged protein, oxidized lipids and peroxides and also to stop chain propagation of peroxyl lipid radical (Table I). These enzymes repair the damage to biomolecules and reconstitute the damaged cell membrane e.g. Lipase, proteases, DNA repair enzymes, transferase, methionine sulfoxide reductase etc.

Cardiovascular Diseases

Epidemiological studies have indicated that vitamins C and E exert protective effect against cardiovascular diseases. Low plasma level or low dietary intake of vitamin C are associated with high blood pressure and unstable coronary syndrome. Taddei et al. concluded that in essential hypertension, vitamin C improves the endothelium dependent vasodilation by increasing type III NOS (eNOS) expression and ensuing NO production. Similarly, Reimersma et al. showed that plasma levels of vitamin E was associated with risk of angina. Vitamin E supplementation reduced non-fatal heart attack, risk of coronary diseases and atherosclerosis by inhibiting oxidation of LDL by free radicals.

Central Nervous System Diseases

Due to high oxygen consumption, low glutathione (antioxidant Status) content, high levels of free iron and oxidizable substances like PUFA in the central nervous system, neuronal cells appear to be particularly vulnerable to oxidative stress. This oxidative stress with decreased antioxidant defense does have a role in the genesis and progression of Alzheimer’s disease, Parkinson’s disease, Brain neoplasmDown syndrome and also some other neurodegenerative disorders.

Carcinogenesis

A major development of carcinogenesis research during the last two decades has been the discovery of significant levels of DNA damage by oxyradicals of endogenous cellular source. These oxyradicals attack DNA causing change in genomic sequences leading to mutation, deletion, gene-amplification or rearrangement. In this manner, oxidative DNA damage was found important in the etiology of many human cancers.
**Inflammatory Diseases**

In chronic inflammatory conditions phagocyte derived ROS have been implicated in inflammation related injury. Intracellular protection of cytoplasmic component against phagocyte derived oxidative injury is mediated predominantly by antioxidant enzymes like SOD, catalase and glutathione peroxidase. The development of mutations in p53 tumour suppressor gene and other key regulatory genes promotes inflammation into chronic disease in rheumatoid arthritis and other inflammatory disorders.

**Renal Diseases**

Free radical injury and oxidative stress have been implicated in many renal diseases like acute renal failure (ARF), IgA nephropathy, anaemia of chronic renal failure (CRF) and ischaemic kidney. Plasma glutathione peroxidase which has been suggested as an index of renal antioxidant defense, is reported to be markedly depressed in CRF and patients undergoing hemodialysis.

**Diabetes Mellitus**

Different studies have given a lot of evidence of increased oxidative stress with depleted antioxidant enzymes and vitamins, in both type 1 and type 2 diabetes mellitus. Hyperglycemia can increase oxidative stress and change the redox potential of glutathione. Now oxidative stress is acknowledged as a pathogenetic mechanism in diabetic complications like diabetic retinopathy, nephropathy, microangiopathy.

**Respiratory Diseases**

Oxidative stress is assumed to play an important role in the pathogenesis of a number of lung diseases, like chronic obstructive pulmonary diseases (COPD), bronchial asthma & acute respiratory distress syndrome, not only through direct injurious effects but also by involvement in the molecular mechanisms that control lung inflammation. Important consequences of oxidative stress in the pathogenesis of COPD include oxidative inactivation of antiproteinas, airspace epithelial injury, increased sequestration of neutrophils in the pulmonary microvasculature and gene expression of proinflammatory mediators.

**Infectious Diseases**

A hallmark of HIV infection is immunodeficiency with progressive CD4+ lymphocytic cell depletion. HIV and opportunistic infections directly or indirectly lead to an oxidative stress, caused by the excess generation of ROS/RNS by activated cells of immune system. Different studies have shown that even in early stages of HIV infection, plasma / lymphocyte SOD level is decreased parallel to the decrease of CD4+ cells number. Another study has shown that HIV Tat protein downregulates MnSOD (mitochondrial SOD) to cause decreased SOD level.

**Aging**

Aging in humans is associated with changes in physical characteristics and the decline of many physiological functions. Increased accumulation of free radicals heightens the vulnerability of older individuals to a variety of oxidative insults. These radicals are capable of causing apoptosis, necrosis and cell death.

**Liver Diseases**

The involvement of free radicals in the pathogenesis of liver injury has been investigated for many years in a few well defined experimental systems. Several studies were conducted to find out the role of oxidant stress and AOS in alcohol induced cirrhosis, which is considered as the terminal irreversible stage of liver diseases. These studies have shown increased lipid peroxidation by HO radical and hydroperoxides in experimental acute and chronic alcoholic liver diseases. Also it has been suggested that ROS and lipid peroxidation may play a role in pathogenesis of hepatic fibrosis with loss of normal liver architecture. In another study it is found that plasma levels of AO vitamins are low in patients with chronic cholestatic liver diseases.

Hepatitis viruses are taxonomically quite diverse and include Picorna, Flavi, Hepadna and calciviruses as well as delta agent associated with hepatitis B virus. Viral hepatitis is caused by both RNA (hepatitis A,C,D,E) viruses and DNA virus (HBV). In these infections, there is an associated enhanced production of ROS/RNS via long term oxidant stress. In another study oxidative stress was observed in peripheral blood mononuclear cells from chronic hepatitis C patients.

The preliminary studies conducted by us at our setup concluded that there is a mild impact of hepatitis viruses in disturbing the antioxidant level in the body. In spite of active diseases caused by hepatitis viruses, the superoxide dismutase and total antioxidant levels in the body do not show significant changes. The behaviour of all the viruses remains similar and does not exacerbate with increasing severity of disease. This means that during viral hepatitis, protective calibre of body against oxidative stress remains intact.
References
11 Karokiriska Institute Press Release for Nobel prize in Physiology or Medicine, December 12, 1998.


