

Free Radicals, Antioxidants and Disease

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Abstract

Living cells continually generate free radicals or reactive oxygen species (ROS) through the respiratory chain during energetic metabolism. ROS can either be harmful or play important physiological roles in our body. Besides being produced during normal cell metabolism there are numerous exogenous factors, such as irradiation by UV light, X-rays, gamma-rays, and atmospheric pollutants which may lead to generation of ROS. Human body has various intrinsic mechanisms to counteract oxidative stress by producing antioxidants, or through externally derived foods and/or supplements. However whenever there is excess of free radicals their accumulation in the body generates a phenomenon called oxidative stress. As we age, this oxidative and/or nitrosative damages elicit a number of late-onset diseases after ROS/RNS accumulate to certain levels. The ROS/RNS-mediated late-onset diseases can occur in any system of the body and may lead to clinical conditions such as cancer, arthritis arteriosclerosis, and neurodegenerative diseases. Oxidative stress is marked with expression of specific biomarkers whose specificity towards the various disease condition needs validation. In this review, we summarize the source, balance, maintenance and physiological functions of ROS, and its toxic mechanisms underlying a number of diseases and also the biomarkers implicated in selected human diseases.

Keywords Free radicals; Antioxidants; Biomarkers; Cancer; Neurodegenerative disorders

Introduction

Oxidative stress is defined as a disturbance in the equilibrium between free radicals (FR), reactive oxygen species (ROS) and the endogenous defense mechanisms [1]. It is the disturbance in the balance between oxidant-antioxidant states which favours the production of oxidant species [2]. Human body requires both oxidant and antioxidant species for normal metabolism, signal transduction and regulation of cellular functions. Therefore, each cell maintains a condition of homeostasis between the oxidant and antioxidant species [3,4]. Oxidative stress may lead to injury to all the important cellular components like proteins, DNA and membrane lipids, which can cause cell death. Oxidative stress has also implicated in various physiological and pathological processes, including DNA damage, proliferation, cell adhesion, and survival which has been validated by several experimental and clinical data in large number of pathological states as well as aging (Figure 1) [2,3].

The broad definition of the ROS is oxygen-containing, reactive chemical species. Up to 1–3% of the pulmonary intake of oxygen by humans is converted into ROS [5]. But it has to be emphasized that ROS and RNS are both produced in a well regulated manner to help maintain homeostasis at the cellular level in the normal healthy tissues and play an important role as signaling molecules. Most cells can produce superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and nitric oxide [NO] when required. Free radicals have several beneficial roles which can be enumerated as:

1. Generation of ATP (universal energy currency) from ADP in the mitochondria: oxidative phosphorylation
2. Detoxification of xenobiotics by Cytochrome P₄₅₀ (oxidizing enzymes)
3. Apoptosis of effete or defective cells
4. Killing of micro-organisms and cancer cells by macrophages and cytotoxic lymphocytes
5. Oxygenases (eg. COX: cyclo-oxygenases, LOX: lipoxygenase) for the generation of prostaglandins and leukotrienes, which have many regulatory functions.

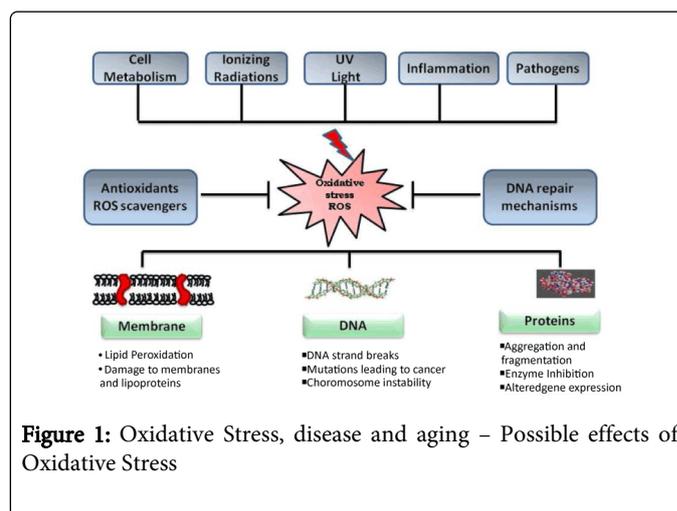


Figure 1: Oxidative Stress, disease and aging – Possible effects of Oxidative Stress

Besides it has also been demonstrated earlier that ROS such as $O_2^{\bullet-}$ and H_2O_2 may act as second messengers and thus can regulate cellular function [4].

Reactive oxygen species (ROS) include superoxide, hydroperoxyl, hydroxyl, alkylperoxyl, alkoxy, carbonate and carbondioxide radicals, while hydrogen peroxide and ozone represent non-radical species (Table 1) [3,5,6]. Nitrogen reactive species (RNS) can be divided into radicals and non-radicals as well (Table 1). Various studies have

demonstrated the role of reactive oxygen species in many degenerative diseases, such as atherosclerosis, cancers, stroke, trauma, asthma, heart attack, hyperoxia, arthritis, age pigments, cataract genesis, retinal damage, dermatitis, liver injury, hepatitis, and periodontitis (Figure 2) [5,6].

Reactive Oxygen Species		Reactive Nitrogen Species	
Free Radicals	Other Substances	Free Radicals	Other Substances
Superoxide anion radical $O_2^{\bullet-}$	Hydrogen peroxide H_2O_2	Nitric oxide radical NO^{\bullet}	Peroxynitrite $ONOO^-$
Hydroxyl radical HO^{\bullet}	Hypochlorous acid $HOCl$	Nitric dioxide radical NO_2^{\bullet}	Nitrites NO_2^-
Alkoxy radical RO^{\bullet}	Ozone O_3		Nitrates NO_3^-
Peroxy radical ROO^{\bullet}	Singlet oxygen 1O_2		Nitrosyl NO^+

Table 1: Summary of reactive oxygen and nitrogen species [5,6]

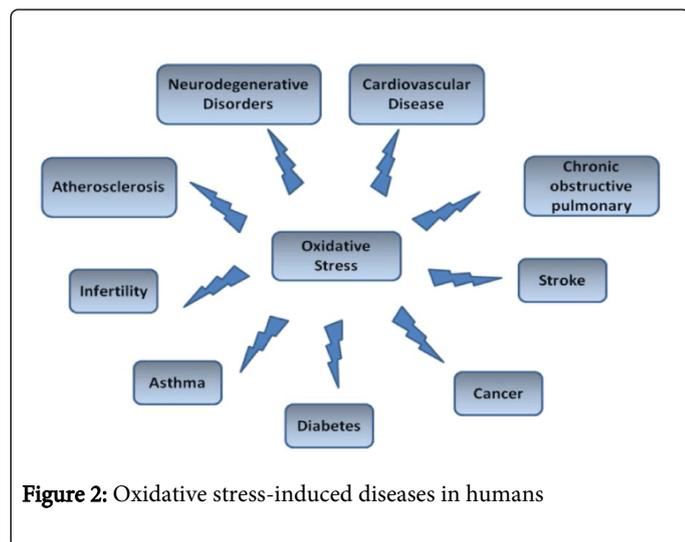


Figure 2: Oxidative stress-induced diseases in humans

Defence System against Free Radicals

Human body has natural antioxidant defence mechanism to counteract the FR produced which when present at very low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate" [7]. The word oxidizable substrate includes almost everything (except H_2O) found in foods such as oil and fat [8]; in living tissues it includes carbohydrates, lipids, proteins, and DNA [7]. There are two major types of antioxidants:

- Synthetic antioxidants: which include the phenolic compounds such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertiary butyl hydroquinone (TBHQ) which are largely used in the food industries to control the oxidation and maintain the food quality [9].
- Natural antioxidants: are the ascorbates, ascorbic acid (Vitamin C), tocopherols, α -tocopherol (Vitamin E), flavonoids (Vitamin P), carotenoids and phenolic acids.

Antioxidants are able to neutralize free radicals at the levels of prevention, interception as well as repair. Antioxidants can stop the formation of ROS for e.g. superoxide dismutase (SOD) catalyses the dismutation of superoxide to H_2O_2 and catalase breaks it down to water [10,11]. Interception of free radicals is mainly by radical scavenging. At the repair and reconstitution level, mainly repair enzymes are involved [10-12] which neutralise the free radicals.

Oxidative Damage to DNA, Lipids and Proteins

The concept of Oxidative stress as first elaborated by Sies et al, 1986 [13] is the ineffective management of free radicals such as ROS and RNS by natural antioxidant defence mechanism thus describing the relation between free radicals and disease (Figure 2). Free radicals mainly attack the cellular components viz. lipids, carbohydrates, proteins and DNA (Figure 1)

Effect on lipids

Lipid components of membrane undergo peroxidation as a result of action of free radicals. During Lipid peroxidation (LP) a large number of toxic by products are also formed that behave as 'second messengers'. The damage caused by LP is highly detrimental to the functioning of the cell [14]. Some of the products of LP such as malondialdehyde (MDA), 4- hydroxynonenal (4-HNE), various 2- alkenals and Isoprostanes are of toxicological interest [14].

Effect on carbohydrates

Carbohydrates are attacked by free radicals such as $\bullet OH$ which randomly abstracts a hydrogen atom from one of the carbon atoms, producing a carbon-centered radical. This phenomenon brings about chain breaks in molecules like hyaluronic acid. Additionally oxyradicals produced as a result of activation of neutrophils during inflammation in the synovial fluid surrounding joints, lead to rheumatoid arthritis.

Effect on proteins

Free radicals can cause direct damage to proteins which can directly interfere with enzyme activity and the function of structural proteins. Oxidation of proteins by ROS/RNS leads to production of stable as well as reactive products such as protein hydroperoxides that can further generate additional free radicals particularly upon interaction with transition metal ions. Mostly these oxidised forms of proteins are rapidly removed however their accumulation over a period of time can contribute to the damage associated with ageing as well as various diseases. Lipofuscin, an aggregate of peroxidized lipids and proteins accumulates in lysosomes of aged cells and brain cells of patients with Alzheimer's disease [15].

Effect on DNA

Free radical attack causes several types of alterations in the DNA such as fragmentation of DNA which in turn causes activation of the poly (ADP-ribose) synthetase enzyme. This splits NAD^+ to aid the repair of DNA. In case of excessive damage NAD^+ levels may become completely depleted leading to cell death which may be by necrosis or apoptosis depending on the type of cellular damage. Damage of cell membrane or an organelle by free radicals makes it vulnerable which may put the entire cell at risk.

Mechanism	References
DNA strand breaks	[21]
Formation of DNA adducts (due to covalent binding)	[21]
Formation of DNA-protein cross-links	[21,22]
Formation of DNA interstrand cross-links	[21]
Activation of oncogenes	[23]
Inactivation of tumour suppressor genes	[23]
DNA base modification and/or destruction	[21,24]

Table 2: Possible mechanisms behind the induction of mutagenesis and carcinogenesis

Oxidative Stress and Disease

Attack of free radicals on the critical cellular components such as lipids carbohydrates, proteins and DNA have been implicated in various pathological conditions such as cardiovascular disease, cancer, neurological disorders, diabetes, ischemia/reperfusion, other diseases and ageing (Figure 2) [16-19].

These diseases can be categorized into two groups: (i) the first group involves diseases characterized by pro-oxidants shifting the thiol/disulphide redox state and impairing glucose tolerance—the so-called “mitochondrial oxidative stress” conditions (cancer and diabetes mellitus); (ii) the second group involves disease characterised by “inflammatory oxidative conditions” and enhanced activity of either NAD[P]H oxidase (leading to atherosclerosis and chronic inflammation) or xanthine oxidase-induced formation of ROS (implicated in ischemia and reperfusion injury). The process of ageing is largely due to the damaging consequence of free radical action (lipid peroxidation, DNA damage, protein oxidation).

Association of oxidative/ nitrosative stress and acute and chronic diseases have been shown by presence of validated biomarkers of oxidative stress [16,20].

Oxidative stress and cancer

Cancer development in humans involves a complex process both at the cellular and molecular levels which is mediated by various endogenous and exogenous stimuli. Reactive species can induce mutagenesis via plenty of possible mechanisms [21-24] (Table 2). Besides carcinogens, radiations can bring about cancer initiation and promotion as a result of free-radical oxidation which is largely in the form of strand breaks in DNA. DNA strand breaks can be both single and double stranded. The breaks are generally repaired, can result in mutations that are heritable change in the DNA which can cause cancer in somatic cells or malformations in the germ cells. Free radicals involvement with tumor suppressor genes and proto-oncogenes indicate their involvement in the development of different human cancers [25]. Additionally lipid peroxidation which plays a key role in controlling cell division, its end product

Malondialdehyde (MDA) due to its high cytotoxic and inhibitory action on protective enzymes further acts as a tumor promoter or a co-carcinogenic agent. This has been shown in some type of cancers such as breast cancer [26]. Besides this oxidative stress may lead to

activation of vascular endothelial growth factor and may induce angiogenesis which may further enhance malignancy [27-29].

Oxidative stress and cardiovascular diseases

Reactive oxygen species (ROS) function as signaling molecules regulating an array of processes in the cardiovascular system and contribute to a large extent in the maintenance of cardiovascular homeostasis [30]. Oxygen free radicals have been reported to play an important role in the pathogenesis of a number of cardiovascular diseases (CVDs) such as atherosclerosis, ischemia, hypertension, cardiomyopathy, cardiac hypertrophy and congestive heart failure by both in vivo and in vitro studies [31-35]. Potential sources of free radicals during ischemia and reperfusion have been identified in myocytes, vascular endothelium, and leukocytes. Injury to processes involved in regulation of the intracellular Ca^{2+} concentration may be a common mechanism underlying both free radical- induced and reperfusion abnormalities [36].

Oxidative stress and neurodegenerative diseases

Oxidative stress and free radical generation catalyzed by redox metals have been shown to play pivotal role in regulating redox reactions in vivo contributing RNS and ROS production which are the, main culprits in neurodegeneration [37]. Mitochondrial (Mt) dysfunctions, excitotoxicity and finally apoptosis are evident causes for neurodegenerative diseases such as Parkinson’s disease (PD), Alzheimer’s disease (AD), Multiple Sclerosis (MS) and Amyotrophic lateral sclerosis (ALS). Mitochondrial dysfunction includes respiratory chain dysfunction and oxidative stress, reduced ATP production, calcium dysregulation, mitochondrial permeability transition pore opening, perturbation in mitochondrial dynamics, and deregulated mitochondrial clearance [38]. The production of β -amyloid, a toxic peptide often found in Alzheimer’s patients’ brain, is due to oxidative stress and plays an important role in the neurodegenerative processes [39]. AD brains also show evidence of ROS mediated-injury; there is an increase in levels of malondialdehyde and 4-hydroxynonenal in brain and cerebrospinal fluid of AD patients compared to controls [40].

In Parkinson’s disease the protein alpha-synuclein (α Syn) binds to ubiquitin and forms proteinaceous cytoplasmic inclusions named Lewy bodies. Over accumulation and post translational modification of α Syn results in death of dopaminergic neurons [41-42]. Besides this increased lipid peroxidation, as well as oxidative DNA and protein damage is observed in substantia nigra [the brain area] that plays a major role in the development of Parkinson’s disease [43-45].

In Huntington’s disease the mutant huntingtin protein (mHtt) aggregates and damage the retrograde transport of important molecules such as BDNF. This damage in transport occurs as a result of damaged molecular motors and microtubules [46] which causes pathological changes and disease symptoms. Additionally altered mitochondrial energy metabolism raises the production of free radicals thus resulting in severe neuronal trauma in Huntington’s disease [47].

In ALS, motor neurons develop proteinaceous inclusions in their cell bodies and axons prior to their destruction. These inclusions generally contain ubiquitin, and often incorporate one of the ALS-associated proteins such as SOD1, TAR DNA binding protein (TDP-43, or TARDBP) or FUS. Protein degradation pathways play a crucial role in removing misfolded proteins thus preventing protein

aggregation. Accumulation of ALS-specific proteinaceous inclusions may be partly due to defects in protein degradation [48]. Evidences from post-mortem tissues from ALS patients have revealed that oxidative stress is the main causative factor responsible for accumulation of oxidative damage to lipids, proteins, and DNA thus suggesting a direct role in ALS progression [47,49,50]. Other disorders associated with oxidative stress and oxidative damage

Cardiovascular, neurodegenerative and oncological diseases are likely to be the most studied pathological conditions associated with oxidative stress. Since oxidation occur in all metabolically active, living cells, and therefore oxidative stress is associated with many other common disorders and conditions (Figure 1). The mechanisms behind the development and progression of such conditions are diverse for e.g. lungs, eyes and skin are naturally exposed to relatively high amounts of oxygen as well as to air pollutants which makes them vulnerable to oxidative damage. Heavy metals such as cadmium increase the generation of reactive oxygen species which promotes cell death [51]. However, the deficiency in exogenous antioxidant defence also plays an important part and can cause problems with ocular tissues [52,53].

Biomarkers

Several biomarkers have been reported as indicators of oxidative stress which include oxidation products of lipid, DNA and protein [16, 54-79] (Table 3). These biomarkers indicate the exposure of various antioxidant protective mechanisms to various oxidants in vivo [50]. Increase in oxidative stress in various pathologic conditions have suggested the use of specific biomarkers for the development of new diagnostic, therapeutic, and preventive strategies for delaying the development of complications such as cancer, atherosclerosis and coronary artery and neurodegenerative diseases.

Concluding Remarks and Future Perspective

Oxidative stress has been implicated in the etiology of several chronic and degenerative diseases [80,81]. Pathological effects of ROS are dealt by human body by utilizing the endogenous antioxidant system (e.g. enzymes such as superoxide dismutase), and by the consumption of antioxidants in the diet (e.g. flavonoids). Insufficient antioxidant levels may accelerate the aging process and some of the diseases associated with it.

The dependence of disease severity by an imbalance between oxidants and natural defenses suggests that antioxidant therapy represents a promising avenue for treatment. However successful development of effective antioxidant therapies still remains a key goal. Many novel approaches have been made and significant findings have come to light in the last few years. The most recent is redox proteomics which is a powerful tool to study redox regulation and signaling which involves global overview of the cellular redox state [82]. The molecular signatures of these short lived ROS/RNS molecules imprinted on lipids and proteins bring about positive oxidative stress, including redox signaling and activation of transcriptional factors [82]. Analysis of the cellular redox state will not only unveil the targets of reactive oxygen and nitrogen species but can also be instrumental in giving valuable insights to counteract oxidative stress. Additionally identification of novel biomarkers specific for disease states arising as a result of oxidative stress will be invaluable in providing information on possible mechanisms of diseases and new potential ways of prevention and treatment.

Type of Biomarker	Disease	References
Ferric reducing ability of plasma	Cardiovascular Diseases	[55]
Carbonyls	AD, Asthma, PD, Diabetes Cardiovascular Diseases	[16,56,75, 76]
Lipid Peroxidation		
Malondialdehyde	AD, Asthma, Atherosclerosis, Cardiovascular Diseases	[57,71,72]
F2-isoprostane	AD, Asthma, Atherosclerosis, Cardiovascular Diseases, Diabetes, Hypertension	[58,73,74]
4-Hydroxynonenal	AD, PD, Atherosclerosis, Cardiovascular Diseases	[16,76]
Plasma vitamins		
Vitamin C	Cardiovascular Diseases	[59]
Vitamin E	Cardiovascular Diseases	[60]
Antioxidant enzymes		
Superoxide dismutase	Cardiovascular Diseases	[61]
Catalase	Cardiovascular Diseases	[62]
Glutathione peroxidase	Cardiovascular Diseases	[63]
GSH/GSSG ratio in erythrocyte	AD, Asthma, Atherosclerosis, Cardiovascular Diseases, Diabetes, PD	[64,77,78]
Prooxidant enzymes		
Xanthine oxidase	Cardiovascular Diseases	[65]
NADPH oxidase	Cardiovascular Diseases	[66]
Others		
Endothelial microparticles	Cardiovascular Diseases	[67]
Endothelial progenitor cells	Cardiovascular Diseases	[68,79]
Ischemia modified albumin	Cardiovascular Diseases	[69]

Table 3: Biomarkers of oxidative stress

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