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Oxidative stress biomarkers in a living cell

Abstract

Living systems are so often exposed to different types of environmental factors leading to the development of stress. Stress is known to exert an adverse impact on the immune system making it weak and susceptible to various diseases. Though the free radicals are produced during the normal metabolism, a delicate balance is maintained between the production level and utilization or removal. With the exposure of living systems to any unfavorable environmental or clinical factors, an imbalance develops between the levels of oxidative and antioxidative species, which leads to the onset of oxidative stress, which is responsible for the occurrence of several severe diseases. Antioxidants are molecules both endogenous and exogenous in nature which neutralize free radicals and save the living system from any damage. In this article, we have summarized in brief an updated account of oxidative stress, factors responsible for it, markers to detect oxidative stress and strategies to restore the cells' vitality to near normal.

Keywords: oxidative stress, free radicals, antioxidants, biomarkers, diseases

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Abbreviations: SOD; superoxide dismutase; GST, glutathione transferases; GPx, glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; ROS, reactive oxygen species; AO, aldehyde oxidase

Introduction

The biomarkers are vital clinical tools used for early diagnosis of any disease and its organ specific location. According to the National Institute of Health, a biomarker is a characteristic parameter that is objectively measured as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.[1] These indicators are determined in the biological fluids like cerebral and spinal fluids, blood, saliva, nasal and vaginal swabs, urine, and tissues.[2] These days, the biomarkers are being very precisely evaluated by employing various diagnostic tools such as proteomics, transcriptomics, metabolomics, and lipidomics.[3] Also, under the lab conditions for acute and sub-acute toxicity tests, the biomarkers can be helpful in the prediction of a specific type of toxicants or xenobiotics duration of exposure, and persistence.[4–6] The list of biomarkers may include biomolecules (glutathione, creatinine, hemoglobin, albumin, uric acid)-, enzymes (transaminases and phosphatases in case of hepatic and cardiovascular diseases; SOD, CAT, GST for oxidative stress), hormones (HCG hormone in pregnancy detection), and antibodies in ELISA, nasal and vaginal swabs for viral/microbial tests for possibility of any pathogenic infections.

The present book chapter illustrates physical, chemical, and behavioural stress mediated over production of free radicals causing varied aberrations in the human health. The excess free radicals result into the development of oxidative stress. This article presents an updated account of the oxidative stress and the role of enzymatic and non-enzymatic antioxidants in maintaining the balance in the redox systems in the body.

Reactive oxidative species (ROS) generation as a principal factor for oxidative stress

The reactive oxygen species (ROS) are normally produced by the ongoing metabolic activities of a living cell for energy and cell survival. NADPH produced or involved in the metabolism is a cofactor necessary to keep cells in a reduced state. It also protects the cell from excessive ROS. Oxidative stress is the major cause of diseases as it significantly reduces the immunity of an individual. The stress can be of any type like heat shock, genotoxic, nutrient, and oxidative stress, etc. Sometimes stresses also develop due to an imbalance in body homeostasis (endogenous) or due to exposure to environmental factors (exogenous).

The exposure to the stress conditions modulates the functions of mitochondrial cytochrome P450 systems resulting in the formation of superoxides, which induce lipid peroxidation, oxidations of proteins, etc. The superoxides are formed due to the incomplete reduction of oxygen to water in the electron transport chain (ETC) and are the main precursors for ROS production. They disturb the reduced state of the cell and hence its normal functions.[7] The excessive production of ROS in a living cell may also be due to certain endogenous factors like improper functioning of defense mechanisms or antioxidants, improper utilization of ROS, or elimination by the scavenging system.[8,9] Exogenous environmental factors like aerosols, UV radiations, heavy metals, microplastics, pesticides, high temperature may cause an increase in ROS production.[10] The ROS may cause irreversible damage to DNA (single/ double strands break, mismatch-/-excision of nucleotides-/-base, cell membrane disruption/membrane permeability, early aging, infertility in males due to oxidation of purine base Guanine.[11,12] ROS can also cause cancer by irreversible activation of cell cycle inhibitors, suppression of tumor suppressor genes p53, Rb genes, and finally cell death. The development of neurodegenerative diseases like Alzheimer's, dementia, and Parkinson's; the cardiovascular diseases due to clogged arteries, inflammations, pulmonary, diabetes, etc. as well as cataracts and many more may occur due to the increased level of oxidative stress. It has been proved that increased oxidative stress also causes the progression and development of HIV, and chronic pancreatitis.[13,14]

However, ROS is not always bad for a cell. It helps in the recruitment of leukocytes at the injured cell or tissue and helps in repair. In a recent review, it has been shown to help in memory formation and storage.[15,16] The ROS formed during exercise improves tissue growth and antioxidants production. It is important in cellular signaling, maintaining homeostasis, and different cellular functions.[17,18] Nano-encapsulated antioxidants prevent antioxidants from endogenous factors of the gut.[19]

Xenobiotics metabolism

Normally 2 phases of xenobiotic metabolism are involved in the detoxification-/- biotransformation of any toxicant/xenobiotics

to remove from the body. In some cases, 3rd phase of xenobiotic metabolism is also involved. Utilizing a battery of specific enzymes, these metabolic phases help transform the hydrophobic compounds to hydrophilic resulting in their removal from the system. The phase 1 reactions are mainly catalyzed by cytochrome P-450 mixed function oxidases in the smooth endoplasmic reticulum of the liver. It either adds oxygen or removes hydrogen from the toxicants making it hydrophilic. Phase 1 metabolism also includes biochemical reactions such as hydroxylation, transamination, epoxidation, racemization, and isomerization, etc. Phase 2 metabolism involves the biotransformation of those xenobiotics, which are not removed by Phase 1. Phase 2 biotransformation reactions involve conjugation reactions. A polar conjugate such as glucuronic acids-/glycine-/sulphate/-GSH is enzymatically added to the xenobiotics. The carboxyl, hydroxyl, amino, and thiol groups of Phase 1 metabolite-/-xenobiotics interact to make a polar conjugate. These conjugates cannot cross the lipids barriers and hence are removed. In Phase 3, the ABC transporters use ATP to remove some hydrophobic anions of the xenobiotics of Phase 2, which are further metabolized and excreted out.[20]

Common factors that aid in oxidative stress and its prevention

Individuals, who are obese, take processed foods, smokers, alcoholics, exposed to toxicants or ionizing radiation, and mines workers are often highly susceptible to oxidative stress diseases. The presence of excess adipocytes (fat storing cells) in obese produce inflammatory substances may enhance ROS production in the body. The most common home remedies and lifestyle changes to reduce stress include eating salads, and fresh foods, regular exercise, walks, drinking plenty of water, quitting smoking, avoiding exposure to toxicants, and meditation.

The vitamins (E, A, C), flavonoids, and glutathione act as normal antioxidants (free radical scavengers).[21–23] They donate an electron to a free radical and to the ROS thereby neutralizing their adverse effects by preventing lipid peroxidation or DNA damage etc. The colored fruits (apples, blue-berries, citrus fruits) and vegetables (lentils, broccoli, and spinach) are very rich sources of antioxidants. Spices like turmeric cumin, clove, ginger, oregano, cinnamon; nuts, seeds, and dried fruits also contain plenty of antioxidants.[24,25]

Some common antioxidative enzymes and non-enzymatic antioxidative biomolecules

Superoxide dismutase

The SOD (EC 1.15.1.1) present in the cytoplasm, mitochondria, and extracellular fluid, protects the cell from superoxides. SOD catalyzes the conversion of superoxide to hydrogen peroxides. SOD has manganese in its active site. H_2O_2 production in excess is very dangerous for cells as it may cause lipid peroxidation and ROS generation. This H_2O_2 is converted to water and oxygen by the action of catalase present in peroxisomes. Increased levels of SOD are seen in chronic periodontitis and cancer.[26, 27] In lichen planus, an autoimmune disease, elevated levels of SOD and decreased level of catalase leading to increased H_2O_2 in the cell has been reported.[28] Mutation in SOD1 or its over-expression is associated with the disease of neurons, and inactivation to hepatocellular carcinoma, whereas its decreased activity is linked to different types of pulmonary diseases.[29-31] Its anti-inflammatory action helps in the treatment of colitis-, and bowel diseases.[32]

Glutathione peroxidase

The glutathione peroxidase (GPx) (EC 1.11.1.9) catalyzes the oxidation of glutathione with H_2O_2. It reduces lipids hydroperoxides (R_2O_2) to alcohols (ROH) and H_2O_2 to water.[33] Its deficiency is associated with diabetic neuropathy, vitiligo, and multiple sclerosis.[34,35]

$$2GSH + H_2O_2 \rightarrow GS-SG + 2H_2O$$

$$2GSH + R_2O_2 \rightarrow GSSG + 2\,ROH\,(R = H,\ alkyl)$$

Peroxiredoxins

The peroxiredoxins (Prx) (EC 1.11.1.15) catalyzes the reduction of H_2O_2, alkyl hydroperoxides, and alkyl nitrites in the nucleus, cytosol, and mitochondria.[36] Prx are the abundant proteins of red blood cells as hemoglobin. Thioredoxins (Trx) can be glutathiones.

$$Reduced\ Prx + H_2O_2 \rightarrow oxidized\ Prx + 2H_2O$$

$$Oxidized\ Prx + reduced\ Trx \rightarrow reduced\ Prx + oxidized\ Trx$$

Glutathione reductase

The glutathione reductase (GR) (EC 1.8.1.7) or Glutathione–disulphide Reductase (GSR) catalyzes the conversion of GSSG to GSH. The reaction uses NADPH and FAD as cofactors. Its activity is monitored by NADPH consumption and its conversion to NADP.[37] It is inhibited by flavonoids.[38] The ratio of GSSG to GSH can give us the exact status of a cell in terms of oxidative stress. It is a phase II enzyme of detoxification reactions. Amide linkage in glutathione protects it from hydrolysis by peptidases.[39] Inactive form of GSR or its deficiency is found in cataract patients. As eye cells have deficient catalase, so H_2O_2 gets accumulated causing oxidative damage to the eye and development of the cataract.[40] Its deficiency causes lysis of RBCs, hyperbilirubinemia and anemia due to the nonfunctional pentose phosphate pathway and increased oxidative stress.[41] It is associated with neutrophils' outburst and release of free radicals to kill the pathogen.[42]

$$NADPH + GSSG + H_2O \rightarrow 2\,GSH + NADP^+ + OH^{-1}$$

$$GS-SG + NADPH + H^+ \rightarrow 2\,GSH + NADP^+$$

Glutathione-S-transferases (GSTx)

The glutathione-S-transferases (GSTx: EC 2.5.1.18) biotransform the toxicants into peroxidized forms which become more water soluble, thereby allowing them to be removed by transporters. The reduced glutathione acts as a nucleophile and attacks electrophilic carbon, sulphur, or nitrogen of non-polar (hydrophobic) toxicants, thereby preventing interactions with cellular proteins and nucleic acids.[43–45] The reduced intracellular oxidative stress was found to be associated with increased GSTs after insulin administration in diabetics, whereas glucagon results in increased oxidative stress and low GSTx.[46] Increased GSTx is associated with liver/ kidney failures or viral infections.[47–48]

Catalase

Catalase (EC 1.11.1.6) protects the cell from oxidative damage by ROS. It converts millions of H_2O_2 formed in a cell to water and oxygen per second.[49] Toxins like formaldehyde, phenols, and alcohols are oxidized by H_2O_2. It is present in peroxisomes and cytosol of red blood cells.

$$H_2O_2 + H_2R \rightarrow 2H_2O + R$$

Copper deficiency reduces its activity in the heart and liver.[50] Persons deficient in catalase are more prone to obesity and type 2 diabetes.[51,52] It interferes with melanin synthesis and so low levels of catalase result in greying of hair because of H_2O_2 accumulation in hair follicles.[53]

Aldehyde oxidase: The aldehyde oxidase (AO, EC:1.2.3.1) is mainly present in the liver and is involved in catalyzing phase 1 xenobiotic metabolism reactions. It works as an oxido-reductase catalysing the oxidation of aromatic aldehydes to carboxylic acids, hydroxylation of immune suppressive drugs, oxidation of cytochrome P450 and nitrogen containing heterocyclic compounds to lactams, and reduction of NO and NS bonds. It requires FAD and molybdenum as cofactors. Due to its broad substrate specificity, AO oxidizes xenobiotics in liver and hepatic clearance.[54]

$$RCHO + H_2O + O_2 \rightleftharpoons RCOO^- + H_2O_2 + H^+$$

Glutathione

Glutathione (GSH), a non-enzymatic antioxidant, is present in a reduced state in the cytosol and other organelles of a living cell. In stressed or diseased conditions, it gets oxidized. In a cell, it exists in 2 different states: reduced (GSH) and oxidized (GSSG). These different forms of glutathione help in quenching free radicals thereby protecting the cell conditions and maintaining its vitality.[55]

Vitamin E (Tocopherol)

It is one of fat-soluble vitamin obtained from diet, among which α tocopherol has antioxidant property. It inhibits lipid peroxidation in membranes, erythrocyte and ROS production. It prevents disease atherosclerosis by decreases the synthesis of prostaglandin (thromboxane) involved in platelet clumping.[56] The proposed mechanism of vitamin E (alpha-tocopherol)-mediated low-density lipoprotein lipid peroxidation adapted from Chan et al.[57]

$$ROO\cdot + \alpha - TOH \rightarrow ROOH + \alpha - TO\cdot$$

$$\alpha - TO\cdot + CoQ10H2 \rightarrow \alpha - TOH + Co\ QH\cdot$$

$$Co\ QH\cdot \rightarrow CoQ$$

$$ROO\cdot + \alpha\ TO\cdot \rightarrow non\ radical\ products$$

$$\alpha - TO + RH\ (PUFA) \rightarrow \alpha - TOH + R\cdot(alkyl\ radical)$$

$$R\cdot + O2 \rightarrow ROO\cdot(peroxyl\ radical\ of\ PUFA)$$

Vitamin C (Ascorbic acid)

It is an essential cofactor for α ketoglutarate dependent dioxygenases like prolyl hydroxylases involved in regulation of genes of cancer, energy metabolism, and apoptosis.[58] This gene in turn is controlled by transcription factor hypoxia induced factor-1 (HIF-1) and vitamin C. Vitamin C availability inhibits the pathway of transcription of HIF. Vitamin C also regulates the function of endothelial nitric oxide synthase by recycling its cofactor tetrahydrobiopterin involved in blood pressure.

Vitamin A (Retinol)

It is a fat-soluble vitamin, essential for vision. It combines with opsin to form rhodopsin necessary for low light and colour vision.[59] Its role has been seen in protection of several diseases of heart, eyes, tumors etc.[60]

Phytochemicals

These are by-products of plants involved in protection from ultraviolet radiations and platelets aggression. They protect us from cancers, neurodegenerative diseases, cardiovascular, osteoporosis etc. The phenol groups in them acts as an electrophile to form stable phenoxy radicals which disrupts ROS formation.[61]

Uric acid: it acts as an antioxidant only in plasma but in cell acts as prooxidant and initiation of several diseases due to oxidative stress like hypertension, visceral obesity, insulin resistance, dyslipidemia, diabetes type II, kidney disease, and cardiovascular, and cerebrovascular events. High concentrations of urate in plasma scavenge hydroxyl, peroxyl radicals.[62]

Melatonin: It is an endogenous hormone secreted from pineal gland. It is synthesized from amino acid tryptophan. Being amphiphilic it enhances the ETC thus reduces electron leakage. As an antioxidant it reduces lipid peroxidation and damage to DNA. It is involved in protection of neurodegenerative diseases like Parkinson's, Alzheimers.[63]

Bilirubin

It acts as antioxidant, but due to its low concentration in nano Moles, can't completely neutralize ROS which is present in milli Moles. High levels of it are neurotoxic but optimum levels are beneficial. Gilbert syndrome, ischemic heart diseases are all due to elevated levels of bilirubin.[64,65]

Albumin

The antioxidant property of human serum albumin relies on the presence of amino acid lysine in its structure. This lysine has high affinity for bilirubin, and their conjugation inhibits lipid peroxidation acting as an indirect antioxidant.[66,67] Its presence in blood prevents leakage of fluids out of the blood vessels.

Ceruloplasmin

It is an α globulin, copper containing ferroxidase that neutralizes the toxicity of ferric ions and converting them to ferrous ions. After this oxidation iron can bind to transferrin. It acts as an antioxidant because of copper which acts as an electron donor and acceptor in iron metabolism. Any mutation or defect in ceruloplasmin leads to iron overload, diabetes mellitus and neurodegenerative diseases.[68]

Ferritin

It acts as an antioxidant by removing the iron when iron overload exists. It acts as a ferroxidase and can take up to 4500 of ferric form of iron in aerobic condition without external oxidase. Its deficiency causes Cooley's Anemia.[69]

Transferrin

It binds to circulating iron which otherwise have taken part in redox reactions. Hence protecting us from ROS formed in free iron overload conditions.[71]

Lactoferrin

It is a reversible iron binding milk protein imparting in reducing iron overload, ROS, and associated diseases. Excess iron also helps in growth of pathogens.[72]

Myoglobin

It is a globular oxygen binding protein produced by myofibrils in skeletal muscle and heart. It acts as a peroxidase (cytochrome C peroxidase) by detoxifying endogenous hydrogen peroxide.[70] Table 1 presents a summary of some common enzymatic and non-enzymatic antioxidants.

Table 1 Common enzymatic and non-enzymatic antioxidants and their functions

Enzymatic antioxidants	Functions	Non-enzymatic antioxidants	Functions
Super Oxide Dismutase	Metalloenzyme providing defense against ROS	Vitamin E (Tocopherol)	Scavenge loose/free electrons that can damage cells
Glutathione reductase	Maintains the supply of reduced glutathione	Vitamin C (Ascorbic acid)	Helps in protecting and keeping cells healthy
Glutathione transferase	Detoxify xenobiotics by Glutathione	Vitamin A (Retinol)	Important for vision, immune cells, growth and reproduction
Catalase	Provides protection from damage by peroxides	Phytochemicals (polyphenols/ Polyamines)	Scavenge toxic radicals generated after oxidative stress/anti-inflammatory, activates autophagy
Peroxiredoxins	Regulates peroxides levels in cells	Glutathione	Promotes detoxification of endogenous and xenobiotics compounds
Oxidases	Carry out the oxidation of CO and CN bonds in oxygen and reduced to hydrogen peroxide	Hydroxyl cinnamic acids	Anti (inflammatory, oxidant, microbial, collagenase, aging)
Glutathione peroxidase	Reduces hydrogen peroxide to water	Uric acid	Stimulates type 2 immune responses
Paraxonase	PON1 helps in drug metabolism, cardiovascular and neurodegenerative diseases	Melatonin	Hormone produced by brain in dark, regulates energy metabolism and glucose homeostasis
Thioredoxin (Trx)	It maintains the reduced environment of the cell by NADPH by converting thioredoxin reductase to thioredoxin	Bilirubin	waste product of erythrocyte degradation, and protect against cardiovascular disease
Aldehyde oxidase	Helps in phase I xenobiotic metabolism	Albumin	Main antioxidant in blood. It chelates metal ions and free radicals
		Ceruloplasmin/ Ferritin/ Transferrin/ Lactoferrin	Acts as antioxidant in decreasing iron overload
		Myoglobin	Oxygen and iron binding proteins in skeletal and cardiac muscles

Conclusion

Development of oxidative stress in an individual due to several reasons including exposure to various environmental factors has been shown to cause cellular damage at the membrane, nucleus, and DNA levels causing the emergence of cardio-vascular, hepatic, renal, neuronal, and immunological disorders. Oxidative stress affects cellular physiology and induces cancer. The rapid and early detection of biomarkers of oxidative stress help understand the metabolic state of the body and disease prognosis, thereby helping the affected individual to start timely treatment.

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Conflicts of interest

Authors declare that there are no conflicts of interest.

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