4 High Altitude-Induced Oxidative Stress, Rheumatoid Arthritis, and Proteomic Alteration

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Abstract

Oxidative stress is the disruption in the equilibrium between the production of pro-oxidants such as peroxynitrite ($ONOO^-$), reactive oxygen species (ROS), reactive nitrogen species (RNS), and superoxide anion ($.O_2^-$), etc. and antioxidants such as catalase, dismutase, etc. Two major sources of oxidative stress are endogenous and exogenous. Enhanced hyperoxia or aerobic metabolism is assumed to have high levels of reactive oxygen and nitrogen species (RONS) that have a high ability to oxidative damage to the lipids, DNA, and protein. High altitude increased the generation of ROS or reduced antioxidants that are the major causes of oxidative damage to macromolecules. Excess supply of oxygen can increase mitochondrial ROS production. In hypoxia, the mitochondrial electron transport system causes the generation of ROS. Short- and long-term exposure to hypoxia can enhance the level of oxidative stress. Rheumatoid arthritis (RA) is a chronic autoimmune condition that can cause joint damage and deterioration of the bone. Oxidative stress in RA includes various causes such as the irregular distribution of adhesive molecules, autophagy induction, and synoviocyte resistance for apoptosis. Several hours of exposure to higher humidity and reduced pressure have a major worse effect on RA. In vivo, ex vivo, and in-cell oxidative stress can be calculated using various instruments such as flow cytometry, fluorescence microplate reader, and confocal

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microscopy, etc. Increased altitude is related to physiological responses to hypobaric hypoxia stress by an increment in oxygen supply and usage of oxygen for tissue via metabolic modulation.

Keywords

Oxidative stress · Rheumatoid arthritis · Hypoxia · High altitude · Reactive species · Proteome

Abbreviations

5-LO	5-Lipoxygenase
AGE	Advanced glycation end product
AOPP	Advanced oxidation of protein products
CL-HPLC	Chemiluminescence-high performance liquid chromatography
CPT1B	Carnitine palmitoyltransferase 1
CT	3-Chlorotyrosine
CYP2E1	Cytochrome P450 2E1
DAF-2DA	Diaminofluorescein diacetate
DCF	Dichlorofluorescein
DCFDA	Dichlorofluorescein diacetate
DHR	Dihydrorhodamine 123
DPPP	Diphenyl-1-pyrenylphosphine
ESR	Electron spin resonance
FAO	Fatty acid oxidation
GSH	Glutathione
HIF	Hypoxia Induce factor
IgG	Immunoglobulin
IR	Ionization Radiation
LDH	Lactate Dehydrogenase
MBL	Mannose-Binding Lectin
MDA	Malondialdehyde
NO	Nitric oxide
NQO	Quinine oxidoreductase
OA	Osteoarthritis
PC	Protein carbonyls
PCOOH	Phosphatidylcholine
PEOOH	Phosphatidylethanolamine
PPARα	Peroxisome proliferator-activated receptor α
RA	Rheumatoid Arthritis
ROS	Reactive Oxygen Species
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
TLRs	Toll-like receptors
TRX	Thioredoxin

4.1 Oxidative Stress

The disturbance in the formation of free radicals and its detoxification by the biological system leads to oxidative stress. Disturbance of the production of antioxidants such as catalase, scavengers, and dismutase, etc. and oxidants such as hydroxyl radicals (.OH), reactive nitrogen species (RNS), superoxide anion ($.O_2^-$), reactive oxygen species (ROS), and peroxynitrite ($ONOO^-$), etc. are referred as oxidative stress. Reactive oxygen species referred as intermediate products of biochemical reactions such as neutrophil-mediated phagocytosis, mitochondrial respiration, and cytochrome P450, etc.

4.1.1 Oxidants

Oxidative stress is a leading cause of the oxidative process and apoptosis, which further leads to cell death. Details of different oxidants are mentioned in Table 4.1. In oxidative phosphorylation, mitochondrial active oxygen leakage is the main source of the production of reactive oxygen radicals. Several redox-active flavoproteins may act as an important factor in oxidant development. Superoxide is produced by various enzymes such as xanthine oxidase, Nicotinamide adenine dinucleotide oxidase (NADPH), and cytochrome P450, etc. Four major endogenous sources of oxidants are: aerobic respiration reduced the O_2 and generate $.O_2$, .OH, and H_2O_2; phagocytosis of bacteria or virus-infected cells generate the O_2^-, nitric oxide (NO), OCl, and H_2O_2; H_2O_2 produced by peroxisome and animal cytochrome P450 generate intermediate products which can damage DNA.

ROS and RNS are known to be involved in the pathogenesis of schizophrenia and Alzheimer's diseases. Oxidative stress can cause hyperoxia, tissue injury, diabetes, and age-related development of cancer. Oxidants are mutagenic and cause DNA

Table 4.1 Different oxidants and their properties

Superoxide anion ($.O_2^-$)	One electron reduction of O_2 forms the $.O_2^-$. It is produced as an intermediate in various auto-oxidative reactions and electron transport chain
Hydrogen peroxide (H_2O_2)	Dismutation of O_2^- forms a two-electron reduction state ROS named as H_2O_2. It can easily cross the plasma membrane due to its lipid solubility
Hydroxyl radical (.OH)	It is produced by the decomposition of peroxynitrite and Fenton reaction. It is three electron reduction state radical and extremely reactive
Organic hydroperoxide (ROOH)	Cellular components like nucleobases and lipids form the ROOH
Peroxynitrite ($ONOO^-$)	Reaction between O^{-2} and $NO^{\cdot}$ produce the peroxynitrite

damage directly or indirectly and may also inhibit apoptosis and facilitate proliferation, invasiveness, and metastasis. Excessive production of vascular O_2 can results in hypertension and vasospasm (Lepoivre et al. 1994). Oxidative stress plays an important role in the break down immunological tolerance, inflammatory processes and can induce apoptosis and even cell death (Dalal et al. 2017; Messner and Imlay 2002; Rice-Evans and Gopinathan 1995; Dalal and Biswas 2019).

4.1.1.1 Endogenous Source

Various intracellular enzymes such as peroxisomes, lipoxygenases, NADPH oxidases, oxidases, etc. can produce the pro-oxidants inside the cells (Landry and Cotter 2014). CYP450 is a heme-containing protein superfamily that can degrade toxic compounds. Dioxygen is activated in the catalytic process CYP450 via a single electron reduction reaction to O_2 in the CYP450 catalytic process (Lewis 2002). Cytochrome P450 2E1 (CYP2E1) can generate ROS in the presence or absence of a substrate, so it is also called leaky enzyme (Robertson et al. 2001).

Lipoxygenase is a metalloenzyme used to metabolize the eicosanoid like leukotrienes and prostaglandins. Arachidonic acid is reduced into 5-Lipoxygenase (5-LO), known to be involved in the formation of leukotriene (Dixon et al. 1990). Leukotriene and 5-LO are directly related to oxidation and inflammation in arthritis, asthma, and neurodegenerative diseases (Joshi and Praticò 2015). Peroxidases are multifunctional enzymes that produce H_2O_2 which is further degraded by catalase (Nordgren and Fransen 2014; Wang et al. 2013). In mitochondria, four-electron transport chain multiprotein complexes (complex I-IV) regulate oxidative phosphorylation and the gradient of electrochemical protons (Liu et al. 2002). Complex I and III interacts with O_2 and release oxygen radical ($O_2^{\cdot}$) in the cytoplasm (St-Pierre et al. 2002).

4.1.1.2 Exogenous Source

Oxidants production can also be controlled by external environmental factors like ionization radiation, bacterial, and fungal toxins and inflammatory cytokines. Exposure of a specific cell type to these external factors may result in ROS that may affect adjacent cells also. The co-expression of multiple Toll-like receptors (TLRs) can cause oxidative stress by disrupting the generation of anti-inflammatory and pro-inflammatory cytokines (Lavieri et al. 2014). It has been reported that ionization radiation (IR) exposure to thyroid cells can generate ROS (Ameziane-El-Hassani et al. 2015). IR is one of the main causes of bonds breakage and generation of free radicals that can contribute to oxidative stress. The induction of *Streptococcus pneumonia*-mediated oxidative stress depends on LytA pneumococcal autolysin (Zahlten et al. 2014). Deoxynivalenol (DON) produced by *Fusarium* can damage the membrane and diminish the cell viability (Yang et al. 2014). DON-treated lymphocytes increase ROS levels, 8-hydroxy-2-deoxyguanosine, and lipid peroxide levels.

4.1.2 Antioxidants

Antioxidants play a major role in counteracting oxidants, which contributes to a further reduction in oxidative stress. Thus, antioxidant and oxidant balance may be used to assess human oxidative stress. Antioxidants possess antitumor, anti-carcinogenic, anti-inflammatory, antibacterial, antiviral, and antiatherosclerotic properties (Owen et al. 2000). Natural antioxidants reduce the risk of diabetes, cancer, and cardiovascular diseases and can, however, cause oxidative DNA damage. Antioxidants can be endogenous or exogenous (natural or synthetic). Both types of antioxidants have the ability to eliminate or scavenge free radicals, which is vital for the generation of ROS. Natural antioxidants can be further diversified into two groups: enzymatic and non-enzymatic, as shown in Fig. 4.1.

4.2 High Altitude Mediated Oxidative Stress

Aerobic metabolism is a necessary consequence for the production of reactive oxygen and nitrogen species (RONS). RONS plays a major role as a physiological or natural modulator of cellular redox milieu, further act as a signal for controlling factors of unknown and known pathophysiological and physiological processes. Increased aerobic metabolism or hyperoxia is generally assumed to easily produce a high level of RONS, that can result in oxidative damage of lipids, DNA, and proteins. Physical exercise over a certain duration or intensity can cause oxidative damage to various organs (Radak et al. 2001).

Nevertheless, the increment in the production level of RONS does not only seem to due to mitochondrial respiration, as the anaerobic activity could also cause oxidative damage (Radak et al. 1998). In addition, the defense of endothelium through the use of superoxide dismutase (SOD) has avoided the oxidative damage of lipids and the activity of xanthine oxidase, which indicates a variety of sources

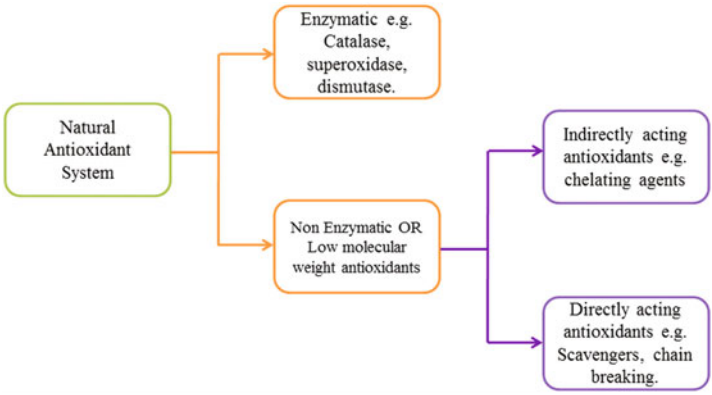

Fig. 4.1 Classification of natural antioxidants

and pathways in the creation of RONS-associated exercise. High altitude toxicity can also cause oxidative damage to macromolecules such as DNA, protein, and lipids. Low oxygen pressure appears to be beneficial for the production of less RONS, but high altitude exposure along with oxidative damage can trigger the RONS production and decrease the antioxidant system activity.

4.2.1 High Altitude and Oxidative Damage

Exposure to intermittent high altitude can significantly enhance the lipid peroxidation in fast and slow muscle fibers of rats (Radak et al. 1994). Radak et al. 1997 did not found an increment in lipid peroxidation after continuous exposure of 4 weeks, however, the amount of protein oxidation detected by carbonyl derivatives was increased (Radák et al. 1997; Kumar et al. 1999). In addition, Nakanishi and colleagues reported the increment in the level of malondialdehyde level in serum, liver, lung, kidney, and heart at an altitude of 5500 m (Nakanishi et al. 1995). It has been reported that 12 healthy subjects to 4559 m of altitude causing major increases in urine-determined DNA strand breaks (Møller et al. 2001). It has been found that simultaneous exposure at a high altitude of 2700 m and cold enhances DNA damage and lipid peroxidation (Schmidt et al. 2002). At 6000 m, the rate of lipid peroxidation rose by 23% and at 8848 m by 79% reveals that the level of oxidative stress is proportional to the rise in altitude (Joanny et al. 2001). Therefore, high altitude causes oxidative damage to proteins, DNA, and lipids by an increment in the level of generation of ROS or a decline in antioxidant capacity.

4.2.2 RONS Generation at High Altitude

The large supply of oxygen can enhance the production level of mitochondrial ROS. It also seems, however, that hypoxia also appears to lead to less stress, which can also result in an increment in the production of ROS (Mohanraj et al. 1998). The reductive stress can increase ROS production by automotive oxidation of mitochondrial complexes. It has been reported that the cellular level of NADH/NAD+ ratio increases during reductive stress (Khan and O'Brien 1995).

During hypoxia conditions, the xanthine dehydrogenase/oxidase system is a powerful ROS generator. High altitude irregular exposure has similar properties to ischemia/reperfusion (Radak et al. 1994). However, the changes in ROS and NO pattern during ischemia/reperfusion and high altitude exposure are different. The initial response is followed by a reversible increment in ROS production during ischemia/reperfusion and is reversed by antioxidants, which can raise the NO in tissue. Unlike ischemia/reperfusion, ROS level rises in hypoxia and return to pre-hypoxic values in normoxia. Acclimatization required inducible NO synthase (iNOS) regulation suggests that hypoxia can alter the ROS/NO balance (Gonzalez and Wood 2001). This phenomenon can influence the microcirculation correlated with acute mountain sickness, hypoxic exposure, high altitude brain edema, and

lung. Serrano et al. reported that the presence of different NOS forms in NO formation at high altitudes might lead to a rise in the production level of nitrotyrosine in rat cerebellum (Serrano et al. 2003).

4.2.3 Hypoxia and Oxidative Stress

The exposure of high altitude can enhance the level of production of ROS and RNS, which can alter the redox balance (Magalhães et al. 2005). It has been shown that hypoxic exposure for short and long term can raise the level of oxidative stress (Joanny et al. 2001; Askew 2002; Dosek et al. 2007). Both types of hypoxia; hypobaric (i.e. terrestrial altitude) and normobaric hypoxia (i.e., simulated altitude) can increase oxidative stress (Magalhães et al. 2005; Debevec et al. 2014). However, hypobaric hypoxia found to triggers a higher level of oxidative stress than normobaric hypoxia (Faiss et al. 2013; Damij et al. 2015; Ribon et al. 2016). Three different mechanisms found to be directly involved in ROS modulation in normobaric and hypobaric hypoxia (Fagerberg 2018). First, hypobaric breathing is lower as compared to normobaric hypoxia along with higher respiratory frequency and lower tidal volume (Faiss et al. 2013; Savourey et al. 2003). In hypobaric hypoxia, higher alveolar physiological dead space is linked with hypocapnia and ventilator alkalosis. Second, higher hypoxemia due to hypobaric hypoxia may also negatively correlated between the level of oxidative stress and hemoglobin oxygen saturation (Bailey et al. 2001). Lastly, hypobaric exhaled NO levels were lower than normobaric hypoxia (Hemmingsson and Linnarsson 2009). Oxidative stress due to environmental hypoxia relies on its duration and intensity (Debevec et al. 2014; Damij et al. 2015). It seems, in general, that major deleterious effects of hypoxia-induced oxidative stress caused by hypoxia are due to high hypoxic doses. It is found that exogenous antioxidant does not appear to mitigate oxidative stress caused by hypoxia throughout exposure at high altitudes (Subudhi et al. 2004).

4.3 Rheumatoid Arthritis (RA)

Rheumatoid arthritis (RA) is a chronic autoimmune disease that proliferates the synovial cells at the joint. A high amount of infiltrates of macrophages, B cells, T cells, and polymorphonuclear cells are found at the inflammatory sites. These cells and cellular factors exhibit a major role in joint destruction in RA. RA involves environmental factors and genetic factors that can activate autoimmune responses. RA is characterized by swelling, heat, inflammation, redness, and joint pain. It causes the proliferation of synovial cells and tissues which is destructive to the cartilage and bone. The autoantibodies present in the serum of RA patients are responsible for the autoimmune reactions in the body.

4.3.1 Oxidative Stress in RA

Macrophage and polymorphonuclear cells play a vital role in the development of ROS by activation of inflammatory molecules that can destroy bones and cartilage in humans (Mapp et al. 1995). In RA, oxidative stress includes many factors such as lymphocytes, autophagy induction, and irregular production of adhesive molecules along with cell damage (Ozkan et al. 2007). Numerous antioxidants such as metallothioneins, thioredoxin, and glutathione (GSH) reductase may be present in RA synovial tissues, but due to their low level, they cannot overcome oxidative stress.

In RA patients, thioredoxin can cause synovial fibroblast cells to produce TNF α induced IL-6 and IL-8. Vitamin C, thiols, and glutathione (GSH) blood levels declined, while Malondialdehyde (MDA) levels in RA patients rose in comparison to the average individual. $O_2^{\cdot-}$, ROS, and HO^- expression levels are elevated in peripheral blood neutrophils and the synovial fluid of RA patients (Kundu et al. 2012). The ROS produced in neutrophils is directly linked to RA (Kundu et al. 2012). Immune cell invasion within RA joints will lead to the creation of RNS/ROS species that further activate the redox responsive pathways, migrate multiple irregular molecules expressed on lymphocytes in synovial fluid of RA patients. Role of oxidative stress in rheumatoid arthritis is shown in Fig. 4.2.

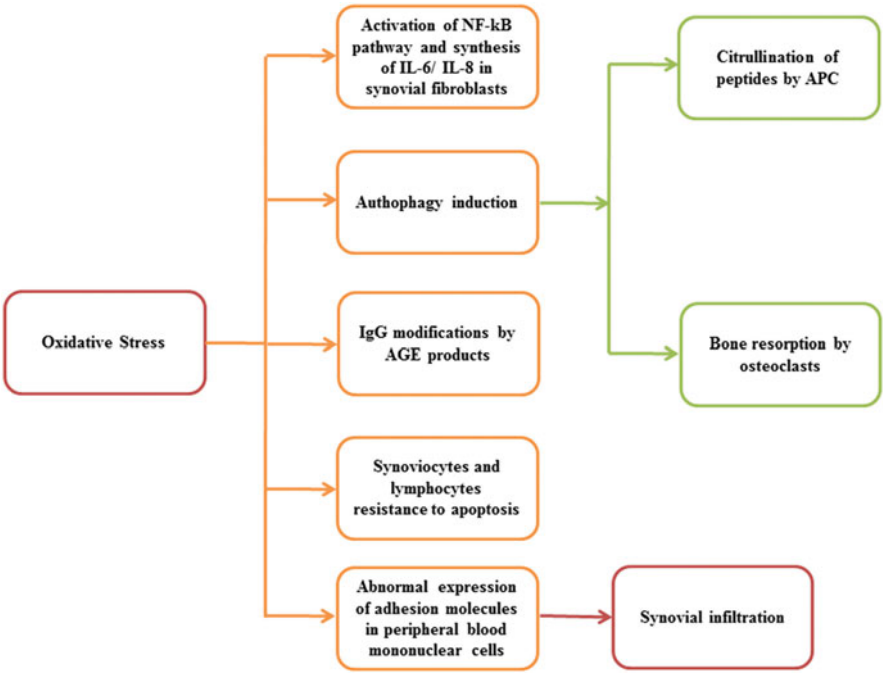

Fig. 4.2 Role of oxidative stress in rheumatoid arthritis

In RA, oxidative stress can cause the modification of immunoglobulin (IgG) (Newkirk et al. 2003). It has been found that advanced glycation end product (AGE) pentosidine and AGE-modified IgG are directly correlated with RA (Kurien and Scofield 2008). Inhibited Caspase 3 activation has not been found in the synovial cells of RA patients (Migita et al. 2001). Induction of autophagy can minimize the apoptosis in the synovial cells of RA patients (Xu et al. 2013). The levels of H_2O_2, thiobarbituric acid reactive substances (TBARS), and O_2 found higher in RA patients as compared to control healthy persons. In RA, the levels of plasma thioredoxin (TRX) and urinary excretion of 8-hydroxydeoxyguanosine (8-OHdG) were higher as compared to healthy controls (Jikimoto et al. 2002).

In RA, ROS generation is known to be directly linked to bone restoration in inflammation processes (Bijlsma and Jacobs 2000). The intracellular ionic environment may be disrupted by hypoxic conditions, which can further alter the levels of calcium and phosphorus (Cheeseman and Slater 1993). In the RA peripheral blood, the rise in lipid peroxidation can induce oxidative stress (Walwadkar et al. 2006). Moreover, lipid peroxidation can generate the MDA, which results in the generation of immunogenic molecules. In RA patients, the level of nitric oxide and lipid peroxide is higher as compared to a healthy individual. While, the concentration of calcium/phosphorus and vitamin E is lower in RA patients than healthy control. The increment in the level of nitric oxide and lipid peroxide and a decline in vitamin E and calcium/phosphorus ratio confirmed the threat of oxidative stress in rheumatoid arthritis. It has been reported that increased oxidative stress induces T cells to trigger various stimuli that can regulate immune responses and even can cause severe problems (Hassan et al. 2011). Thirty different antioxidants and oxidants were identified in RA patients. These were classified as: (1) lipid peroxidation, (2) protein oxidation, (3) DNA damage, (4) urate oxidation, (5) enzymatic activity, (6) antioxidants, and (7) free radical/anions (Quiñonez-Flores et al. 2016).

4.3.2 High Altitude and RA

It has been reported that climatic changes have a worsening effect on arthritis (Holbrook 1960). In RA, the period after the storm or rainfall is most painful (Singh et al. 1977). It has been found that a few hour exposures of rising in humidity and a decline in pressure can have a significant worsening effect on RA (Hollander and Yeostros 1963). Higher humidity and dropping barometric pressure are followed by intracellular fluid diuresis and extrusion of intracellular fluid into the blood. Diseased tissues lost its permeability, retain fluids, and therefore maintain higher intracellular pressure as compared to surrounding tissue, which further results in increased pain and swelling. Due to this, RA patients benefit from the warm and dry Southwest climate of the USA (Singh et al. 1977). It has been reported that patients at 35% humidity and 32 °C also improved (Edström 1944).

Men exposed to a dry and alternate warm and cold environment does not have adversely affect due to enhanced immune response and fibrinolytic activity rather than from meteorological variations. The excess of deposition of fibrin results in

inflammation in RA, which further causes an elevation in the deposition of more fibrinogen (Fearnley et al. 1966). Exposure of high altitude can enhance the excretion of 17-hydroxysteriod in the urine which can elevate the synthesis of corticosteroids that may play a pivotal role in the prevention of RA.

4.3.3 Reactive Species Measurement in RA

Several reports show that different biomarkers like advanced oxidation of protein products (AOPP), 3-Chlorotyrosine (CT), and nitrosothiols can be used for protein oxidation evaluation in RA (Datta et al. 2014; Stamp et al. 2012; Tetik et al. 2010). It has been found that the rate of carbonylation of protein is higher in the plasma samples of RA as compared to healthy (Stamp et al. 2012; Tetik et al. 2010). In RA patients, protein carbonyls (PC), AOPP, and RNS have been determined (Datta et al. 2014). It has been reported that the level of 3-Chlorotyrosine (CT) is more in RA than a healthy person (Nzeusseu Toukap et al. 2014). Malondialdehyde (MDA) is the end product of lipid peroxidized decomposition reactions. It has been found that the MDA level is enhanced in the synovial fluid of RA patients (Gambhir et al. 1997). The fluorometric method can be utilized to measure plasma lipid peroxidation level in RA (Conti et al. 1991).

The activity of GSH-Px can be determined by spectrophotometric at 37 °C and 412 nm (Gambhir et al. 1997). The spectrophotometer can be used to measure the hydrogen peroxide and molybdate at 405 nm (Gambhir et al. 1997). The levels of antioxidants in plasma of RA can be measured by automated calorimetric methods (Erel 2004). The intracellular NO can be measured by a non-fluorescent dye diaminofluorescein diacetate (DAF-2DA), which fluorescent after reaction with NO (Sarkar et al. 2011).

4.4 Oxidative Stress Measurement

Various techniques such as HPLC, GC-MS, UV-spectroscopy, and immunoassays can be used to determine the concentration of principal biomarkers of lipid, DNA, and protein oxidative damage, as shown in Table 4.2.

The level of oxidative stress can be calculated by detecting the concentration of RS. RS can be measured Ex vivo, in vivo, or inside cells. Various techniques such as L-band electron spin along with nitroxy probe and magnetic resonance imaging spin can be used to detect RS directly inside the cells (Berliner et al. 2001). RS can be measured directly or indirectly by measuring the concentration of generated $NO^{\cdot -}$ and $H_2O_2^{\cdot}$ RS can be detected by measuring the concentration of trapped species or oxidative damage.

Electron spin resonance (ESR) can measure the free radicals directly by detecting the unpaired electrons. However, reactive radicals cannot be detected by ESR as they do not accumulate to a sufficient level for measurement. This problem was solved with the introduction of probes or trap agents that can form stable reactive radicals

Table 4.2 Principal markers of oxidative stress and their detection techniques

Markers	Techniques	Matrices
8-Oxo-guanine	GC-MS	DNA
	HPLC-ECD	Urine, DNA
8-oxo-2′-deoxy-guanosine	HPLC-ECD	Urine, DNA
5-(hydroxymethyl) uracil	GC	DNA, synthesized oligonucleotides
8-hydroxy-deoxy-guanosine	HPLC ECD	Urine, DNA
Hydroperoxides	Enzymatic methods	Plasma
	HPLC-MS	Plasma
	HPLC-CL	Tissue, plasma, cellular membranes
	Iodometric methods	Plasma, cellular membranes
	GC-MS	Cellular membranes
Isoprostanes	Immunoassay	Urine
	GC-MS	Plasma, tissue, urine
Malondialdehyde	HPLC	Plasma
	TBA test	Plasma, serum, tissue
4-hydroxynonenal	HPLC	Plasma, tissue
	GC-MS	Plasma, tissue, urine
Malondialdehyde	HPLC	Plasma
	TBA test	Plasma, serum, tissue
	GC-MS	Plasma, serum, tissue
Isoprostanes	Immunoassay	Urine
	GC-MS	Plasma, tissue, urine
	Radioimmunoassay	Plasma, urine
4-hydroxynonenal	HPLC	Plasma, tissue
	GC-MS	Plasma, tissue, urine

along with unstable radicals that can be detected easily by ESR. Spin traps use the hydroxylamine probes to detect the free radicals in the liver and skin (Haywood et al. 1999). It can detect the generation of secondary radicals such as lipid produced (peroxyl, alkoxyl, etc.) and protein radicals also. Aromatic traps, including phenylalanine and salicylate, are more effective than spin traps (Ingelman-Sundberg et al. 1991). Salicylate and phenylalanine can be used to assess the development of radical in RA patients (Liu et al. 1997). In Saliva, phenylalanine was used to detect the concentration of the generation of $OH^{\cdot}$.

Dichlorofluorescein diacetate (DCFDA) is widely used for the detection of cellular peroxidases, although it interacts very slowly with lipid peroxidases or H_2O_2 (Ischiropoulos et al. 1999). DCFDA converts into dichlorofluorescein (DCF) which can be seen at 525 nm. Dihydrorhodamine 123 (DHR) was used to detect NO_2, $OH^{\cdot}$, and $ONOO^{-}$, etc., although it reacts poorly to NO., O_2^{-}., and H_2O_2 (Buxser et al. 1999). DHR converted into rhodamine123 is fluorescent at 536 nm. Dihydroethidium is oxidized into a fluorescent product (ethidium) that can fluoresce at 600 nm after excitation at 500–530 nm (Fig. 4.3) (Zhao et al. 2003). Ethidium can detect the $O_2^{\cdot -}$ and intercalate into nuclear DNA.

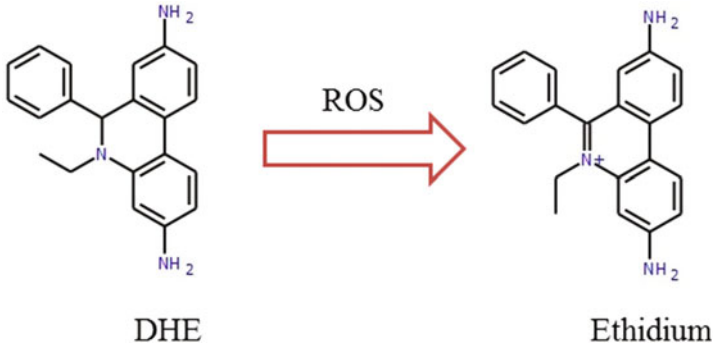

Fig. 4.3 Conversion of DHE to Ethidium

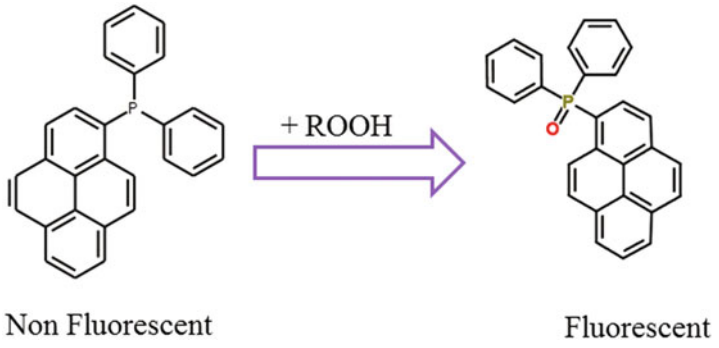

Fig. 4.4 Conversion of diphenyl-1-pyrenylphosphine (non-fluorescent) to a fluorescent product

Luminol can be used to measure the concentration of RS developed through phagocytosis activation (Faulkner and Fridovich 1993). However, luminol cannot measure $O_2^{\cdot-}$ directly, it reacts with $O_2^{\cdot-}$ and generate the fluorescent product. Diphenyl-1-pyrenylphosphine (DPPP) can react to peroxides and make the fluorescent product that can be detected at 380 nm after excitation at 351 nm (Fig. 4.4) (Takahashi et al. 2001). Lipid soluble hydroxide can react with Diphenyl-1-pyrenylphosphine while it cannot react with hydrogen peroxides. The fluorescent DPPP is quite stable in living cells and remained up to 2 days, whereas other minor effects such as cell morphology, proliferation, or cell viability remained up to 3 days.

Fluorescence plate reader is the simplest method that measures the variation in fluorescence. However, the machine's sensitivity and efficiency vary enormously, and the addition of extra filters and other parts will make it costly. Flow cytometry provides the benefit of measuring the cellular culture directly by fluorescence. Quantitative data can be collected on the number of cells that emits fluorescence at a certain wavelength. Although it has a drawback, the addition of trypsin can result in the development of oxidative stress. In mouse liver, the concentration of phosphatidylethanolamine (PEOOH) and phosphatidylcholine (PCOOH) was calculated by

chemiluminescence-high performance liquid chromatography (CL-HPLC) (Miyazawa et al. 1987). Lipid peroxidation produce the endoperoxides, hydroperoxides, and final products: ethane, pentane, so it is the most accurate method of determination of ROS. It has been reported that determination of concentration of PEOOH or PCOOH is one of the most accurate methods of lipid peroxidation analysis (Miyazawa et al. 1987).

4.5 High Altitude and Proteomic Alteration

Exposure of hypobaric hypoxia increases the mandatory biological processes that can enhance the oxygen delivery along with cardiac output, ventilation, and hematocrit in lowlanders rising to the altitude (Peacock 1998). Similarly, physiological traits that can increase oxygen flux have been selected in high altitude populations (Beall 2007). However, there is a pattern of acclimatization, which may vary between highland populations such as Tibetans has higher resting ventilation rates as compared to Andeans, while arterial oxygen contents and hematocrits are lower than Andeans or lowlanders (Beall 2007). Exhaled vasodilator nitric oxide and signal molecules are higher in Andeans as compared to lowlanders and Tibetans (Beall et al. 2001). Several variants in the GTP-cyclohydrolase 1 gene involved in the stabilization of NO synthase have been enhanced in Tibetans with high circulating NO levels. Furthermore, NO can promote pulmonary perfusion and provide protection for pulmonary hypertension as experienced at altitude by outlanders (Busch et al. 2001). In Tibetan, elevated circulatory NO metabolites are also linked to the increased flux of limb blood flow and NO itself can lead to the modulation of hematocrit, reducing blood viscosity (Erzurum et al. 2007; Ashmore et al. 2014).

A genomic analysis in the Tibetan highlanders demonstrated a peroxisome proliferator-activated receptor (PPARA) haplotype positively selected and correlated with the phenotype of a lower hematocrit (Simonson et al. 2010). Peroxisome proliferator-activated receptor α (PPARα) encoded by PPARA, which plays an important role in the regulation of cell metabolism. PPARα is expressed in liver, heart, and muscle and can enhance the expression of fatty acid metabolism controlling genes (Gulick et al. 1994; Gilde and Van Bilsen 2003). PPARA haplotype is correlated with an increment of non-esterified fatty acids, which can result in a decline of whole-body fatty acid oxidation (FAO) in Tibetan (Ge et al. 2012). Whereas, in Sherpas, PPARA haplotype is co-related with a decrease in the expression of skeletal muscle PPARα and carnitine palmitoyltransferase 1 (CPT1B) which can result in a decrease in mitochondrial FAO capacity (Horscroft et al. 2017). In hypoxia, cellular oxygen requirements may be reduced due to switch in the substrate of ATP synthesis from fatty acid to non-fatty acids. It has been reported that reduction in FAO capacity and increment in mitochondrial coupling efficiency after some time of altitude exposure in native lowlanders make them adaptable (Horscroft et al. 2017; Jacobs et al. 2012).

The increment in glycolytic flux in highlander and lowlander populations can activate hypoxia induce factor (HIF) to induce lactate efflux and glycolysis in the cells (Semenza et al. 1994; Kim et al. 2006; Papandreou et al. 2006). Enhancement in lactate dehydrogenase (LDH) activity indicates the increment in cardiac glucose uptake and lactate efflux capacity in Sherpas as compared to lowlanders (Horscroft et al. 2017; Holden et al. 1995). Therefore, increment in glucose metabolism, especially glycolysis is a function of adaptation and acclimatization to high altitude.

4.6 Conclusion

The disturbance between the production of antioxidants and oxidants in a biological system can result in oxidative stress. Oxidative stress is one leading cause of apoptosis which can cause in cell death. Four oxidants development sources are: aerobic respiration, phagocytosis of bacteria or virus, H_2O_2 production by peroxisome, and cytochrome P450. Oxidants are mutagenic in nature and play a major role in invasiveness, metastasis or suppression of apoptosis. Arachidonic acid is reduced into 5-LO which can cause inflammation in asthma, arthritis, and neurodegenerative conditions. Specific environmental factors, such as ionizing radiations, bacterial and fungal toxins, and inflammatory cytokines, can also play an important role in the regulation of development of oxidants. Antioxidants are required to counter the oxidants which can decrease oxidative stress. Antioxidants exhibit antitumor, anti-carcinogenic, anti-inflammatory, antibacterial, and antiviral properties.

The development of reactive oxygen and nitrogen species required an aerobic metabolism process. Increment in hyperoxia or aerobic metabolism can produce a high level of RONS, which can result in oxidative stress. Physical exercise after a certain intensity or duration can cause oxidative damage to several organs. Exposure of high altitude can increase the rate of lipid peroxidation in fast and slow muscle fibers. Even short exposure at high altitudes can increase lipid peroxidation. An abundant supply of oxygen can enhance the development of mitochondrial ROS.

Rheumatoid arthritis (RA) is a chronic autoimmune condition that proliferates in the articulation of the synovial cells. Autoantibodies in the serum of RA patients are responsible for the body's autoimmune reactions. Rheumatoid factor is known as an autoantibody for RA diagnosis, however, it is present in two-third patients only. Several other antibodies, such as heterogeneous nuclear RNPs, mannose-binding lectin (MBL), and immunoglobulin binding protein (BiP) can also be used for the detection of RA. Polymorphonuclear cells and macrophages may induce ROS, the generation of ROS can trigger chronic inflammation which can destruct the human bone and cartilage. The reduction in antioxidants levels of blood increases the chance of RA development. A few hours of elevated humidity and reduction in pressure will greatly exacerbate RA effects. Oxidative stress in RA can be measured by protein oxidation or lipid oxidation by detection of various biomarkers such as AOPP, RSNO, CT, and MDA, etc.

Several techniques such as GC-MS, HPLC, and UV-spectroscopy, etc. can be used to measure the concentration of reactive species. Reactive cell species can also be detected by using different compounds such as Dihydrorhodamine 123 (DHR), Diphenyl-1-pyrenylphosphine (DPPP), or luminol, etc. Different techniques such as flow cytometry, confocal microscopy, and fluorescence microplate reader, etc. have been used to detect the oxidative by measurement of the production of reactive species. Physiological acclimatization can be observed in lowlanders at altitude. These differences between individuals are due to genetic difference among them. Hypobaric hypoxia can enhance the biological process, resulting in an increment in cardiac output, ventilation, and hematocrit. Increased altitude can alter the expression or activity of various proteins such as PPARA, LDH, HIF, and CPT1B, etc.

In the last decade, research has been done on the detection of free radicals. Several techniques, along with sensors or probes, have been identified. There is a requirement of the development of new sensors or probes to measure the ROS within a human cell. The production of molecules that can inhibit oxidants or activate antioxidants is highly required. New biomarkers are required to detect the proteomics alteration at high altitude.

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