Markers of oxidative stress in erythrocytes and plasma during aging in humans

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Aging is an inevitable universal biological process, which can be characterized by a general decline in physiological function with the accumulation of diverse adverse changes and increased probability of death. Among several theories, oxidative stress/free radical theory offers the best mechanistic elucidation of the aging process and other age-related phenomenon. In the present paper, we discuss the aging process and have focused on the importance of some reliable markers of oxidative stress which may be used as biomarkers of the aging process.

Introduction

Aging is a complex progressive physiological alteration of the organism which ultimately leads to death. Observational and descriptive studies performed on a large variety of aging systems have demonstrated that the main molecular characteristic of aging is the progressive accumulation of damages in macromolecules. Although different types of molecular damage accumulate at different rates and to different extents in different cells, the fact remains that there is a progressive increase in molecular heterogeneity with age. There are many ways to define aging and gerontologists are struggling for years to come to an agreement due to the complexity of the aging process. The aging process induces age related changes and leads to increased occurrence of many diseases. Aged individuals exhibit an inherited susceptibility to various degenerative diseases and appear less able than their young and adult counterparts to withstand physiological stress. Thus broadly, aging may be defined as an inherently complex process that is manifested within an organism at genetic, molecular, cellular, organ and system levels.

Although the fundamental mechanisms are still poorly understood, a growing body of evidence points toward the oxidative damage caused by reactive oxygen species (ROS) as one of the primary determinant of aging. A certain amount of oxidative damage takes place even under normal conditions, however the rate of this damage increases during the pathological conditions like diabetes, cardiovascular diseases and cancer. An increased oxidative stress has been linked to a shortening of life span.

Free Radical/Oxidative Stress Theory of Aging

There are several theories which attempt to explain the process of aging including caloric restriction, telomere theory and evolutionary theory. The oxidative stress hypothesis/free radical theory of aging proposed in 1956, updated in 2006 by Harman, offers the best mechanistic elucidation of the aging process and other age-related events. ROS are produced by aerobic cells as a byproduct of their metabolic processes as well as mitochondrial respiration. These ROS exist in the cell in balance with antioxidant molecules. A condition of oxidative stress occurs when this critical balance is disrupted due to depletion of antioxidants or excess accumulation of ROS or both i.e., when antioxidants are depleted and/or if the formation of ROS increases beyond the ability of the defenses to cope, then oxidative stress occurs and causes its detrimental effects.
consequences. Such stress occurs when severely adverse environmental or physiological conditions overwhelm biological systems. Thus one rapid and clear indicator of oxidative stress is the induction of antioxidant defenses and/or increases in endogenous ROS levels.

ROS production and accumulation is a common denominator in many diseases and environmental insults and can lead to severe cellular damage leading to physiological dysfunction and cell death in virtually all aerobes. When oxidative stress occurs, cells function to counteract the oxidant effects and to restore redox balance by resetting critical homeostatic parameters. Such cellular activity leads to activation or silencing of genes encoding defensive enzymes, transcription factors and structural proteins. According to the free radical theory of aging, oxidative stress increases with increasing age, this condition leads to accumulation of oxidation products of lipids, nucleic acids, proteins, sugars and sterols ultimately causing cellular dysfunction and making body prone to external deleterious agents (Fig. 1).

In present review we evaluate some reliable and reproducible biomarkers of oxidative stress in human plasma and erythrocytes which are being increasingly used in studies monitoring redox changes with age. The review provides current knowledge of the use of erythrocytes as model cells for the study of aging.

### Erythrocytes and Plasma: Model System of Human Aging

Though many model systems are frequently used to study the biochemical alterations during aging as well as the condition of oxidative stress including the tissues from various parts of the body, erythrocytes or red blood cells get superiority amongst them. Erythrocytes are the most common type of blood cells and are the vertebrate body’s principal means of delivering oxygen from the lungs or gills to body tissues via the blood (Fig. 2). Red blood cell along with its membrane has always been an important medium for the study due to the important role it plays in varied physiological and metabolic processes. Erythrocyte has been increasingly studied because it is the easiest available human cell type. The erythrocyte has been modified by evolutionary forces into a highly specialized cell that is mainly responsible for providing oxygen to tissues and partly excrete carbon dioxide from waste. Red blood cells are capable of extreme changes in shape. Due to their flexibility, red blood cells can easily squeeze through capillaries much narrower than their diameter and can recover rapidly to their original shape. Mature red blood cell is a biconcave disc shaped with a diameter of 8 micron and thickness of 2 micron having an average life span of 120 ± 20 days. Due to the absence of cell organelles particularly nucleus and mitochondria, red blood cell loses its ability to synthesize amino acids and fatty acids and thus red blood cell have a limited capacity of metabolism barely enough to survive its life span. Red blood cells are continuously being removed from the circulation and to maintain their adequate number they are regularly being formed from the bone marrow in accordance with their rate of removal by reticulo-endothelial cell.

There is no dispute that erythrocytes have a definite lifespan in all animal species investigated so far, and there is little dispute on the length of the species-specific lifespan. It implies that, as in other cells, life and death are well regulated for erythrocytes, in spite of their lack of capacity for protein synthesis. During aging the erythrocyte volume decreases with time, with an increase in density especially during the first, and a decrease in hemoglobin
content especially during the second part of the lifespan.16 These changes are associated with a loss of cholesterol and phospholipid and a linear decrease in the mean surface area of 20%, which all point towards a loss of membrane constituents during aging. This loss could be readily explained through the formation of microvesicles by erythrocytes of all ages with vesicles derived from older erythrocytes containing more hemoglobin. Immunological analysis of these vesicles by flow cytometry and immunoblotting revealed that a portion, but not all of these vesicles contain IgG at their surface, expose phosphatidylserine, and may contain breakdown products of band 3 that are also found on the old erythrocytes. High performance liquid chromatography (HPLC) analysis showed that the hemoglobin composition of these vesicles resembles that of the old erythrocytes.17

Biochemical Parameters of Aging

During the whole life the organism is confronted with oxidative stress on one side from intrinsic origins as the mitochondrial power generation is leaking ROS/reactive nitrogen species (RNS) and on the other side from extrinsic origins.18,19 Various methods on diverse systems have been documented to study the various markers of oxidative stress and aging. However most of the biomarkers get influenced by several factors such as life style, nutrition and types of models. Less reproducibility adds another drawback to consider most of the parameters as marker of stress. For these reasons there is a critical need for well-validated parameters that yield absolute quantitative data that can be used on multiple systems. The widespread application of such techniques would provide valuable data on the relative importance of different oxidants, and information of the absolute importance of damage to different target biomolecules. In present section we have explained only those parameters which are most reliable, reproducible and provide a correct assessment of the extent of oxidative stress.

Reducing Power/Antioxidant Capacity of Plasma

Antioxidant capacity of plasma is the primary measure and marker to evaluate the state and potential of oxidative stress in aging and other aging-induced pathological events such as diabetes, cardiovascular diseases and cancer. Since imbalance between antioxidant and oxidants generates the condition of oxidative stress, estimation of the reducing power/antioxidant capacity plasma is the first step in the prediction of oxidative stress in aging process. A number of methods has been used to measure total antioxidant capacity in vitro. These methods are mostly based on quenching of stable free radicals such as 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH•),20 2,2-azobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) by antioxidants;21 inhibition of lipid peroxidation;22 fluorimetric measurement of h-phycoerythrin (in the oxygen radical absorbance capacity assay (ORAC)).23 These assays are useful due to their sensitivity and minimal sample preparation and thus are widely used in biochemical analysis of clinical samples.24 However; most of them are time consuming and utilize expensive chemicals and/or instrumentation. The FRAP (ferric reducing ability of plasma) assay developed by Benzie and Strain25...

![Diagram: Development of oxidative stress in erythrocytes. Hb: hemoglobin, SOD: superoxide dismutase, CAT: catalase. Under normal conditions, reactive oxygen radicals are buffered by endogenous defensive enzymes i.e., superoxide dismutase and catalase but due to reduced reducing/antioxidant capacity during aging or in other pathological conditions, reactive oxygen radicals escape and destroy the macromolecules, which ultimately results in altered erythrocytic behavior.](image)
gets superiority because it is not dependent on the enzymatic/non-enzymatic method to generate free radical prior to evaluation of the anti-radical activity of plasma. The FRAP assay offers a putative index of antioxidant capacity and reducing potential of biological fluids and is simple, convenient, less time consuming and reproducible.

The correlation between antioxidant capacity and oxidative damage during aging has been reported in several tissues in different species, however data on changes of oxidative stress markers in plasma and erythrocytes of healthy populations during aging are few and sometimes contradictory. A study carried out in our laboratory on 80 normal healthy subjects of both genders between the ages of 18 and 85 years, showed that there was a significant decrease in reducing potential of plasma as a function of human age. Many factors including dietary polyphenols influence the extent of reducing/antioxidant potential plasma. In 1998, Cao and co-workers investigated the responses in serum total antioxidant capacity following consumption of strawberries (240 g), spinach (294 g), red wine (300 ml) or vitamin C (1.250 mg) in eight elderly women. The results showed that the total antioxidant capacity of serum determined as ORAC, trolox equivalent antioxidant capacity (TEAC) and FRAP, using the area under the curve, increased significantly by 7–25% during the 4 h period following consumption of red wine, strawberries, vitamin C or spinach. Ferric reducing ability of plasma, as an index of total antioxidant capacity of plasma was found to be enhanced significantly in suckling rats pretreated either with vitamin K1 or menadione (vitamin K3) at a dose of 15 mg/kg b.w.3 days as reported by Hadi et al. The effect of vitamin K1 on FRAP was dose-dependent and it was inversely related to the formation of lipid peroxidation products in plasma as judged by thiobarbituric acid reacting substances (TBARS). These results suggest that the drug-related induction in FRAP occurs only in immature animals as a part of protective mechanism against lipid peroxidation products generated in plasma. Recently Li and co-workers reported a decrease in total antioxidant capacity and effect of synthetic oligosaccharides on antioxidant enzymes and lipid peroxidation in aged mice. Thus measurement of the reducing/antioxidant potential is the primary biochemical approach towards evaluation of oxidative stress during aging.

**Extent of Erythrocyte Lipid Peroxidation**

Lipids are the important constituents of membranes and function as steroid hormones, retinoic acids and prostaglandins. Unstable carbon radicals from fatty acids can rearrange to short alkanes and conjugated dienes which are exhaled or react with oxygen further to peroxyl radicals and finally by hydrogen abstraction to result in lipid hydroperoxides (Fig. 3). The first oxidation products are limited in their function as they are either volatile or highly reactive. Some of them can easily react to secondary oxidation products like malondialdeyhde (MDA), 4-hydroxy-2,3-trans-nonenal (HNE), isoprostanes or oxysterols. These secondary oxidation products influence gene expression and protein synthesis and these can lead to further damage by cross linking proteins.

Lipid peroxidation was first studied in relation to the deterioration of foods in 1930s, when the study on the chemistry of free radical reactions made remarkable advancements. With increasing evidence showing the involvement of free radicals in biology, lipid peroxidation has received renewed attention from wider viewpoints in the fields of chemistry, biochemistry, nutrition and medicine. Later studies revealed that just like proteins, carbohydrates and nucleic acids, lipids are targets of various reactive oxygen and nitrogen species and oxidized to give a diverse array of products. Studies have revealed that lipid peroxidation severely affects biomembranes. It induces disturbance of fine structures, alteration of integrity, fluidity, permeability and functional loss of biomembranes and also modifies low density lipoprotein (LDL) to proatherogenic and proinflammatory forms, and generates potentially toxic products. Lipid peroxidation products have also been shown to be mutagenic and carcinogenic and has been implicated in the underlying mechanisms in numerous disorders and diseases such as cardiovascular diseases, cancer, neurological disorders and also in aging.

One of the most often used biomarker to investigate the oxidative damage on lipids is the measurement of MDA, the major lipid peroxidation product, this can react with the free amino group of proteins, phospholipids and nucleic acids leading to structural modification, which can induce dysfunction of immune systems. A high level of lipid peroxidation products can be detected in cell degradation after cell injury or disease. Lipid peroxidation is a free-radical chain reaction which is accelerated by ROS. Cell membranes are phospholipid bilayers with extrinsic proteins and are direct targets of lipid oxidation. As lipid oxidation of cell membranes increases, the polarity of lipid-phase surface charge and the formation of protein oligomers increase, and molecular mobility of lipids, number of SH (thiol) groups, and resistance to thermo-denaturation decrease. Recent findings have emphasized the importance of lipid peroxidation in relation to the role of caloric restriction and the extension of longevity. Long-lived mammals and birds possess low degrees of unsaturation in their cellular membranes; this leads to lower levels of lipid peroxidation and lipoxidation-derived protein modification in long-lived species.

The measurement of MDA according to the method of Esterbauer and Cheeseman is very easy to perform, fast as well as cheap. The erythrocyte membrane is prone to lipid peroxidation under oxidative stress that involves cleavage of polyunsaturated fatty acids at their double bonds leading to the formation of MDA. The study carried out in our laboratory on a group of normal healthy young, middle and old aged people of both sexes, showed a significant positive correlation between the erythrocyte MDA level and human age. Similar age-dependent increase in erythrocyte MDA has also been reported for European subjects; however, we find that the increase in MDA in the Indian population is much greater than that reported in European subjects. This observation is strengthened by the finding that healthy older free-living European subjects do not appear to be exposed to an acute oxidative stress.
In the continuation of study, the correlation of antioxidant activity of plasma of the same volunteers has been also compared with the MDA concentration. The increase in MDA correlated significantly with decrease in the antioxidant capacity of the plasma during human aging, confirming that the decrease in antioxidant power in body is a reason for increased oxidative stress during aging.\(^4\) Since the measurement of MDA is easy and fast to perform and the results are very reproducible, it is one of the most important biomarkers for the evaluation of the status of lipid peroxidation and is a biomarker of aging.

### Protein Oxidation

The main molecular characteristic of aging is the progressive accumulation of damages in macromolecules. Proteins are likely to be major targets, as a result of their abundance in cells (proteins compose approximately 70% of the dry mass of most cells), plasma, and most tissues, and their rapid rates of reaction both with many radicals and with other oxidants.\(^1\) Proteins are one of the major categories of age-related damage in macromolecules, which have been observed during various cells, tissues and organs. ROS can lead to oxidation of amino acid residue side chains, formation of protein-protein cross-linkages, and oxidation of the protein backbone resulting in protein fragmentation.\(^2\) One of the greatest challenges in aging research today is the identification of specific cause of oxidative modification and accumulation of macromolecules specifically proteins. Oxidative damage leads to loss in specific protein function, since proteins have unique biological functions, there are often unique functional consequences resulting from their modification. It is estimated that almost every third protein in a cell of older animals is dysfunctional as enzyme or structural protein due to oxidative damage.\(^3\) Therefore, the measurement of the protein oxidation is a clinically important factor for the prediction of the aging process and age-related diseases.

Oxidation of proteins can lead to a whole variety of amino acid modifications. Action of chloraminated oxidants, mainly hypochlorous acid and chloramines, produced by myeloperoxidase in activated neutrophils, forms dityrosine containing cross-linked protein products known as advanced oxidation protein products (AOPPs) and are also considered as biomarkers to estimate the degree of oxidative modifications of proteins.\(^4\) The attack of ROS against proteins modifies amino acids; lysine, arginine, proline and histidine residues generating carbonyl moieties, which has been identified as an early marker for protein oxidation and is used as a measure of protein damage.\(^5\) Generation of protein carbonyl derivatives occurs by oxidative modifications of proteins either by the \(\alpha\)-amidation pathway or by oxidation of glutamyl side chains, which leads to formation of a peptide in which the N-terminal amino acid is blocked by an \(\alpha\)-ketoacyl derivative. However, direct oxidation of lysine, arginine, proline and threonine residues may also yield carbonyl derivatives.\(^6\) In addition, carbonyl groups may be introduced into proteins by reactions with aldehydes (4-hydroxy-2-nonenal, malondialdehyde) produced during lipid peroxidation\(^7\) or with reactive carbonyl derivatives (ketoamines, ketoaldehydes, deoxysozones) generated as a consequence of the reaction of reducing sugars or their oxidation products with lysine residues of proteins.\(^8\)

Some protein damage is reversible, such as peroxiredoxin inactivation, methionine sulfoxide formation, s-nitrosylation. Other damage, for example oxidation of side-chains to carbonyl residues, appears irreversible and the protein is destroyed.\(^9\) The presence of carbonyl groups in proteins has therefore been used as a marker of ROS-mediated protein oxidation. Erythrocytes are highly susceptible to
oxidative damage due to the high cellular concentration of oxygen and hemoglobin, a potentially powerful promoter for the oxidative processes. Recently we have shown an increased level of protein carbonyls in human erythrocyte membrane during oxidative stress condition. An age related increase in the protein carbonyl concentration was reported for tissues like heart, muscle or brain as well as in plasma of healthy people. 

Except protein carbonyls, AOPPs, branched-chain amino acids and lipofuscin are other markers of oxidative damage of proteins moieties however use of protein carbonyls as index of oxidative stress has some advantages in comparison with the measurement of other oxidation products because of the relative early formation and the relative stability of carboxylated proteins. Age dependent increase in protein carbonyl level is direct evidence towards the fact that oxidative stress increases during aging, as the antioxidant capacity of the body falls. Although, proteolytic systems exist to remove oxidized proteins, protein oxidation is still an excellent biomarker of oxidative stress due to the relative long half-life of such oxidized proteins. Following severe oxidative stress, the decrease in the proteolytic degradation and accumulation of mis-folded proteins may be the cause and/or the consequence of many disorders and aging.

Reduced Glutathione and Membrane -SH Groups

Reduced glutathione (GSH) is a major intracellular non-protein -SH compound and is accepted as the most important intracellular hydrophilic antioxidant. GSH has many biological functions, including nutrient metabolism, and regulation of cellular events such as gene expression, DNA and protein synthesis, cell proliferation and apoptosis, signal transduction, cytokine production and immune response, and protein glutathionylation. GSH also plays a role in maintenance of membrane protein -SH groups in the reduced form, the oxidation of which can otherwise cause altered cellular structure and function. Glutathione deficiency has been reported in many diseases such as Kwashiorkor, Alzheimer disease, Parkinson disease, liver disease, cystic fibrosis, sickle cell anemia, AIDS, cancer, heart attack, stroke and diabetes. Glutathione system is the most important endogenous defense system against oxidative stress in body. Under oxidative conditions GSH is reversibly oxidized to glutathione disulfide (GSSG). A recent study on age-related changes in GSH in rat brain suggests a significant age-related reduction in the GSH level in all regions of the brain, associated with an increase in GSH oxidation to GSSG and decrease in the GSH/GSSG ratio. The GSH/GSSG redox, per se, functions in redox signaling and control as well as antioxidant protection. The thiol/disulfide redox states may provide central parameters to link environmental influences and progression of changes associated with aging.

GSH plays a key role in protecting cells against electrophiles and free radicals. This is due to the nucleophilicity of the -SH group and to the high reaction rate of thiols with free radicals. Stress resistance of many cells is associated with high intracellular levels of GSH. Cells containing low levels of GSH were found to be more much more sensitive to the effect of irradiation and stress than controls. Indeed, GSH can act directly as a free radical scavenger by neutralizing HO•, or indirectly by repairing initial damage to macromolecules inflicted by HO•. There is ample evidence that thiols protect molecules from radiation injury, mainly by hydrogen donation, which can restore damaged molecules to their original state. Moreover, GSH is a substrate or a cofactor for a number of protective enzymes, such as GSH peroxidase, the GSH S-transferases, or the glayoxalase.

Different studies have pointed out the importance of determination of blood glutathione for both pathological and physiological purposes. Herebergs et al. have reported that cancer patients are more likely to respond to treatment if their erythrocyte GSH and, by inference, tumor GSH concentrations are low. There are alterations of GSH reductase activity in peripheral-blood erythrocytes in such diseases as hypothyroidism and riboflavin deficiency. Exhaustive physical exercise causes changes in the glutathione status of blood, liver and muscles.

A recent study on Indian populations of different age groups revealed an age-dependent decline in intracellular-reduced GSH concentration which correlates with the total antioxidant capacity of the plasma. Gil et al. reported a slow but significant decline of the intra erythrocytic GSH concentration with aging in European subjects. Similar observations have been reported by Erdem-Inal et al.; however, another study on European subjects did not detect any change in GSH levels. Human erythrocytes are rich in -SH functions; the importance of the erythrocyte -SH group in overall cellular redox balance has been emphasized.

Membrane oxidative damage has a considerable effect on membrane mechanical properties. Membrane -SH group oxidative damage may be an important molecular mechanism inducing changes in the membrane microelasticity or whole cell deformability under conditions of physiological and pathological oxidative stress. The altered redox balance with increasing age may affect the activity of virtually any protein since essentially all proteins contain cysteine (Cys) and methionine (Met), amino acids that are subject to oxidation reduction changes. Studies from our lab revealed that both, the reduced glutathione and membrane -SH group are important markers of oxidative stress and it was reported that their concentrations are significantly low in higher age group humans in comparison to younger as well as middle aged humans.
carnitine, cholesterol, amino acids and certain peptide hormones. Due to such a vital role in physiology and urinary loss of this vitamin, make it necessary to maintain high intracellular ASC concentration. ASC has also been shown to protect membrane and other hydrophobic compartments from oxidative damage by regenerating the antioxidant form of vitamin E. Ascorbate can recycle α-tocopherol in low-density lipoprotein (LDL) in the face of an oxidant stress and thus affords protection against oxidation. Thus, recycling of ASC also helps to protect α-tocopherol and serves to protect the erythrocyte against transmembrane oxidant stress. Recently it was reported that in human erythrocytes the activity of PMRS along with the AFR reductase increases with the increase in age. This activation of PMRS and AFR reductase increases the ASC recycling in human plasma and reported as a compensatory/protective mechanism that operates to maintain the ASC level in plasma and thereby minimize oxidative stress during aging.

PMRS, also includes coenzyme Q (CoQ) and increasingly recognized as a...
Antioxidative Enzymatic Systems

Several enzymes have evolved in aerobic cells to overcome the damaging effects of ROS. They are significantly used to maintain the redox balance during oxidative stress and are collectively called as endogenous antioxidative enzymes. Superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT) are the main endogenous enzymatic defense systems of all aerobic cells. They give protection by directly scavenging superoxide radicals and hydrogen peroxide, converting them to less reactive species. SOD catalyzes the dismutation of superoxide radical (•O₂⁻) to hydrogen peroxide (H₂O₂). Although H₂O₂ is not a radical, it is rapidly converted by fenton reaction into •OH radical which is very reactive. GPx neutralizes hydrogen peroxide by taking hydrogens from two GSH molecules resulting in two H₂O and one GSSG. GR then regenerates GSH from GSSG. CAT, the important part of enzymatic defense, neutralizes H₂O₂ into H₂O.

Figure 5. Superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT) are the main endogenous enzymatic defense systems of all aerobic cells. They give protection by directly scavenging superoxide radicals and hydrogen peroxide, converting them to less reactive species. SOD catalyzes the dismutation of superoxide radical (•O₂⁻) to hydrogen peroxide (H₂O₂). Although H₂O₂ is not a radical, it is rapidly converted by fenton reaction into •OH radical which is very reactive. GPx neutralizes hydrogen peroxide by taking hydrogens from two GSH molecules resulting in two H₂O and one GSSG. GR then regenerates GSH from GSSG. CAT, the important part of enzymatic defense, neutralizes H₂O₂ into H₂O.

A very interesting mechanism shown by the CAT depending upon the concentration of H₂O₂ is its dual function. CAT from ROS. The liver, in particular, is very high in SOD. Cellular concentration of SOD relative to metabolic activity is a very good lifespan predictor of animal species. Most mammals experience a lifetime energy expenditure of 200,000 calories per gram, but humans have an amazing 800,000 calories per gram. Humans have the highest levels of SOD relative to metabolic rate of all species studied. Oxidative damage to DNA is ten times greater in rats than in humans. Maximum lifespan correlates with lower rate of free-radical production and higher rate of DNA repair.

The SOD molecule in the cytoplasm contains copper & zinc atoms (Cu/Zn-SOD), whereas the SOD in mitochondria contains manganese (Mn-SOD). SODs have been isolated and characterized from a wide variety of organisms. One class consists of SODs with Cu(II) plus Zn(II) at the active site (Cu/ZnSOD), another with Mn(III) (MnSOD), a third with Fe(III) (FeSOD), and a fourth with Ni(II/III) (NiSOD). Cu/ZnSODs are generally found in the cytosol of eukaryotic cells, in chloroplasts, and in some prokaryotes.

Glutathione peroxidase (GPx) neutralizes hydrogen peroxide by taking hydrogens from two GSH molecules resulting in two H₂O and one GSSG. The enzyme glutathione reductase then regenerates GSH from GSSG. CAT, the important part of enzymatic defense, neutralizes H₂O₂ into H₂O.
neutralizes the \( \text{H}_2\text{O}_2 \) in concentration dependent manner. At low concentrations (<1 \( \mu \text{M} \)) of \( \text{H}_2\text{O}_2 \), it acts “peroxidatically”, i.e., a variety of hydrogen donors (e.g., ethanol, ascorbic acid) can be oxidized in the following manner:

\[
\text{RH}_2 + \text{H}_2\text{O}_2 \rightarrow R + 2\text{H}_2\text{O},
\]

while at higher concentrations of substrate, CAT decomposes toxic hydrogen peroxide at an extremely rapid rate using the “catalatic” reaction in which \( \text{H}_2\text{O}_2 \) acts as both an acceptor and donor of hydrogen molecules:

\[
2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2.
\]

Another property that makes CAT unique among \( \text{H}_2\text{O}_2 \) degrading enzymes is that it degrades \( \text{H}_2\text{O}_2 \) without consuming cellular reducing equivalents. Hence, CAT provides the cell with a very energy efficient mechanism to remove hydrogen peroxide. Therefore, when cells are stressed for energy and are rapidly generating \( \text{H}_2\text{O}_2 \) through “emergency” catabolic processes, \( \text{H}_2\text{O}_2 \) is degraded by CAT in an energy-efficient manner. This results in a net gain of reducing equivalents and, therefore cellular energy. It has been proposed that CAT may be uniquely suited to regulate the homeostasis of \( \text{H}_2\text{O}_2 \) in the cell. Therefore activity of CAT is one of the important biomarker of oxidative stress.

There is a suggestion that the activity of antioxidant enzymes may play an important role in determining the life span of animal species. Most of the workers have shown the decreased activities of these enzymes during oxidative stress as well aging, however the activity especially in regard to SOD is still in controversy. Some researches could not detect an age related difference or they showed an increase in age. These discrepancies can be in part related to the fact that humans express different SODs. Gianni et al. discovered that there is no difference in total activity or CuZn-SOD activity while the activity of Mn-SOD increases with age. Same but less results have also been observed in the case of activity of CAT. Many investigations showed that the activity of CAT increases with age. While some investigations oppose this finding and reported no significant change in the CAT activity. The effect of aging and oxidative stress on the glutathione reductase (GR) and glutathione S-transferase (GSH-S-T) was very clear where negative correlations with age were reported. This corresponds with a shift of the glutathione system to the oxidized form.

A recent study on human plasma of Indian population has shown a significant correlation between increasing age and activity of CAT and SOD in human plasma. The activity of CAT and SOD was increased in the samples of old aged groups in comparison of the young aged samples. Interestingly a positive correlation was found between the antioxidant capacity of the plasma and activities of CAT and SOD. The increased activity of antioxidant enzymes among aged persons seems to be a compensatory mechanism against high levels of ROS in old age; perhaps this adaptability ensures that oxidation-mediated damage takes place at a rate that determines the pace at which we age, defined by Harman as the ‘inborn aging process’.

**Conclusion**

The deleterious effects of oxidative stress generated by uncontrolled production of ROS/RNS play a very crucial role in onset of aging. Various biochemical parameters get directly affected and altered during aging (Fig. 6). Several parameters are being used to evaluate the extent of oxidative damage, however not all the parameters can be used as biomarker of aging process because many of them get influenced by several factors including sex, types of tissue, diet and also their efficient repairing. Meanwhile some parameters are also dependent upon the methods used to measure them. An increasing body of evidence points to the importance of erythrocytes to act as model cells for the study of aging and age-related diseases. The erythrocyte provides an array of biochemical parameters which have been successfully used to assess aging related changes in redox status.

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