Oxidative Stress Biomarkers

Subjects: Biology

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Oxidative stress biomarkers can be used to detect oxidative damages occurring concomitantly in many cellular structures, which may cause a deterioration of function, including apoptosis and necrosis. There is an intimate relationship between oxidative stress, inflammation, and functional impairment, resulting in various diseases affecting the entire human body.

Keywords: oxidative stress ; biomarkers ; antioxidants ; lipid peroxidation ; protein peroxidation ; DNA peroxidation ; signaling pathway ; kidney ; renal transplantation ; ischemia-reperfusion injury

1. Introduction

Reactive oxygen species are compounds that are difficult to measure when assessing oxidative stress, primarily due to the very short half-life, so they hardly play the role of biomarkers. However, if ROS combines with a particular biological molecule, it leaves a unique chemical “fingerprint”. Biomarkers obtained that way can be used to evaluate oxidative damage or the effects of antioxidants, including therapeutic agents. The core criterion for the biomarker is its role in the prediction of the later development of disease. Moreover, important technical criteria of a biomarker are it should detect a major part of total ongoing oxidative damage in vivo, should provide coherent laboratory assays, results should not vary under the same conditions, should be stable during storage, must employ chemically robust measurement technology, and must not be confounded by diet [1]. There is no ideal biomarker, yet many provide sufficient accuracy. ROS, as highly reactive substances, interact with the environment in vivo, involving and stimulating various endogenous mechanisms as well as react with numerous molecules, leaving a mentioned fingerprint, which becomes the point of interest in specific evaluations. ROS, reactions, and essential antioxidants were presented in **Figure 1**.

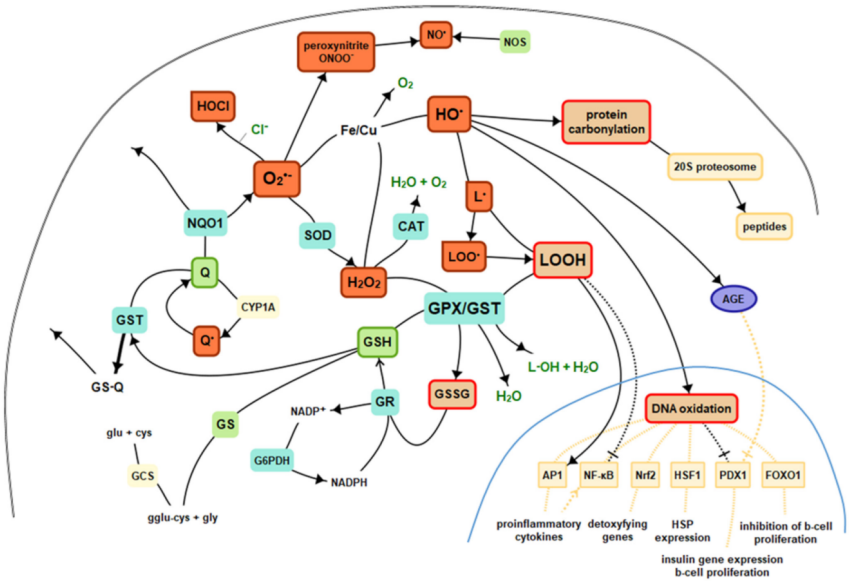


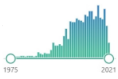
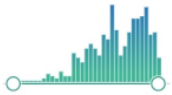
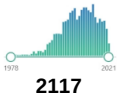
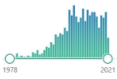
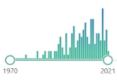
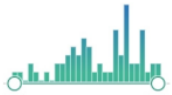
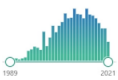
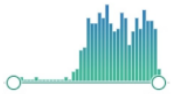
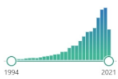
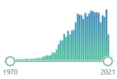
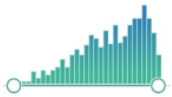
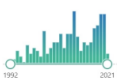
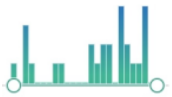
Figure 1. Basics of intracellular antioxidant mechanisms and nuclear signaling. Legend: CAT—catalase, GPx—glutathione peroxidase, GR—glutathione reductase, GST—glutathione S-transferases, GSH—reduced glutathione, GSSG—oxidized glutathione, Q—coenzyme Q_{10}, GS—glutathione synthetase, HO·—hydroxyl radical, H_2O_2—hydrogen peroxide, L·—alkyl radical, LO·—alkoxy radical, LOO·—peroxy radical, HOCl—hypochlorous acid, NO—nitric oxide, NOS—nitric oxide synthase, NQO1—NADPH-quinone oxidoreductase-1, O_2—molecular oxygen, $O_2^{\bullet-}$—superoxide, SOD—superoxide dismutase, AGE—advanced glycation end products, NF-κB—nuclear factor kappa-light-chain-enhancer of activated B cells, AP1—activator protein 1, Nrf2—nuclear factor-erythroid 2-related factor 2, HSF1—heat shock factor 1, PDX1—insulin promoter factor 1, FoxO—forkhead transcription factor O.

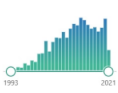
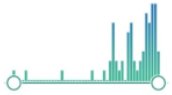
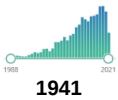
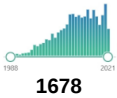
2. Oxidative Stress Biomarkers

2.1. Endogenous Antioxidants

ATP cell production is inherently connected with oxidation, reduction, and ROS generation. External factors involve microbial infections, xenobiotics, diet toxins, radiation, environmental pollution, and others. Living organisms developed specific defense systems against the deleterious action of free radicals. The most important mechanisms are intracellular; however, they act with both extracellular and dietary exogenous antioxidants. Endogenous antioxidants are divided into two groups: protein (with enzymatic activity) and non-protein. Protein ones are the first line of defense, with three the most important: CAT, SOD, and GPx. PubMed search formula for general interest was determined by: {("biomarker"[Title/Abstract]) AND (oxidative stress)}. Research interest in the field of transplantation, as a Medical Subject Headings, was determined by search formula: {("biomarker" [Title/Abstract]) AND (transplantation)} (**Table 1**).

Table 1. Endogenous antioxidants—description and research interest.

Biomarker	Description	General	Transplantation
CAT catalase	**tetramer, enzyme, biomarker, catalyzes the decomposition of hydrogen peroxide to water and oxygen, has one of the highest turnover numbers of all enzymes, first noticed in 1818**	 **1068**	 **356**
SOD superoxide dismutase	**enzyme, biomarker, metalloprotein connected in humans with Zn/Cu or Mn, discovered in 1968**	 **2117**	 **715**
GPx glutathione peroxidase	**selenium-containing enzyme family, several isozymes are encoded by different genes, biomarker, the protective system depends heavily on the presence of selenium, discovered in 1957**	 **763**	 **296**
GST glutathione S-transferase	**family of metabolic isoenzymes, biomarker, catalyze the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates, nomenclature first proposed in 1992**	 **104**	 **95**
GR glutathione reductase	**homodimer disulfide oxidoreductase enzyme encoded by the GSR gene, biomarker, catalyzes GSSH and regenerates it to GSH, involves NADPH and FAD binding domains, first purified in 1955**	 **159**	 **63**
HO-1 heme oxygenase 1	**an enzyme, mediates the first step of heme catabolism, cleaves heme to biliverdin, carbon monoxide, and ferrous iron, encoded by HMOX1 gene induced in oxidative stress and inflammation, first characterized in 1962**	 **1457**	 **404**
NQO1 NADPH-quinone oxidoreductase-1	**protein homodimer, detoxifying enzyme, present in cytosol, performs a two-electron reduction of quinones to hydroquinones and of other redox dyes without the formation of ROS,**	 **1837**	 **86**
GSH glutathione	**L-γ-glutamyl-L-cysteinyl-glycine, non-enzymatic, biomarker, reduced form (GSH) and glutathione disulfide (GSSG)—primary redox couple in animal cells**	 **5118**	 **798**
coenzyme Q ubiquinone	**benzoquinone derivate, localized in the mitochondrial respiratory chain and other internal membranes, coenzyme, first isolate was in 1950 in the lining of a horse's gut**	 **185**	 **22**

Biomarker	Description	General	Transplantation
ALA α-lipoic acid	**delivers antioxidant activity in nonpolar and polar mediums, effective in recharging enzymes in the mitochondria, might counteract NF-κB activation**	 1430	 62
BR bilirubin	**product of heme degradation works as an antioxidant in cycle BR-biliverdin, relevantly documented in 1827**	 1941	 64
ferritin	**intracellular globular iron-binding protein, small amounts are secreted into the serum, prevents ROS generation via the Fenton reaction by binding iron**	 1678	 60

2.2. Lipid Peroxidation (LPO)

Lipids can be oxidized, chlorinated, and nitrated by a range of RS, excluding H_2O_2, $NO^{\bullet}$, or $O_2^{\bullet-}$, which are unreactive with lipids. Lipid peroxidation is a complex process, and a wide range of products is formed in variable amounts.

It is the most widely known biological free radical chain (FRC) reaction. The oxidation of unsaturated fatty acids or other lipids results in products, which are peroxides of these compounds. Note that ROS does not initiate peroxidation. Their presence only intensifies the process. Peroxidation reaction, like every FRC, can be divided into three stages:

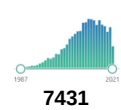
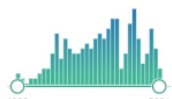
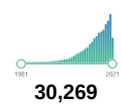
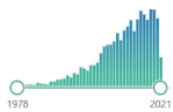
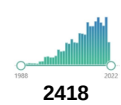
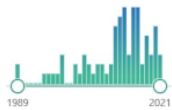
1. Initiation: Creation of fatty acid radical. ROS which initiates this reaction in living cells are hydroxyl (HO·), peroxy (LOO·), alkoxy (LO·), and alkyl (L·), as well as O_3, SO_2, and NO_2. The separation of hydrogen leads to the formation of an alkyl radical. LH $\rightarrow L^{\bullet} + H_2O$.

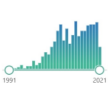
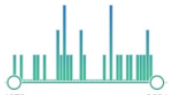
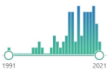
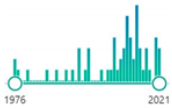
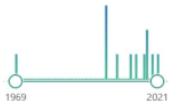
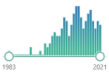
2. Prolongation: Unstable fatty acid radicals easily react with molecular oxygen (O_2), forming peroxides, which are also unstable and react with more fatty acid molecules, creating more radicals. The reaction runs in a cycle: $L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$ $LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet}$.

3. Termination: Growing number of free radicals increases the probability of collision between them, which ends the process: $L^{\bullet} + L^{\bullet} \rightarrow L–L$, $LOO^{\bullet} + LOO^{\bullet} \rightarrow L=O + LOH + O_2$, $LOO^{\bullet} + L^{\bullet} \rightarrow L=O + LOH$. Termination reaction results in products such as dimers of fatty acids, hydroxy acids, and oxoacids.

In **Table 2**, we summarized biomarkers of lipid peroxidation (BLP) with histograms of research interest. General interest was determined by search formula: {("biomarker"[Title/Abstract]) AND (oxidative stress)}. Research interest in the field of transplantation, as a Medical Subject Headings, was determined by search formula: {("biomarker"[Title/Abstract]) AND (transplantation)}. In the last decade, there was a significant growing interest in MDA, with proportional interest in transplantation. However much less expressed, two other "popular" were TBARS and 4-HNE with similar reflection in transplantation. Surprisingly there was a little interest in isoprostanes in transplantation.

Table 2. Biomarkers of lipid peroxidation—description and research interest.

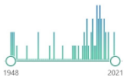
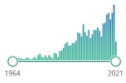
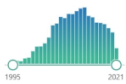
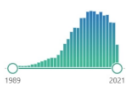
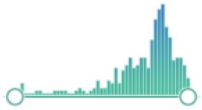
Biomarker	Description	General	Transplantation
TBARS TBA-reactive substances	**the oldest and one of the most widely used nonspecific by-products of lipid peroxidation, reacts with thiobarbituric acid (TBA), forming a pink chromogen (TBARS) measured at 532–535 nm**	 7431	 200
MDA malondialdehyde	**$CH_2(CHO)_2$, colorless liquid, highly reactive, a product of LPO of polyunsaturated fatty acids, form covalent protein adducts referred to as advanced lipoxidation end-products (ALE), in analogy to advanced glycation end-products (AGE)**	 30,269	 2808
4-HNE 4-hydroxynonenal	**α,β-unsaturated hydroxyalkenal, produced by lipid peroxidation (arachidonic or linoleic groups) in cells in higher quantities during oxidative stress, possible role in cell signal transduction, first reported in 1991 [2], they can also come from omega-3 fatty acids**	 2418	 73

Biomarker	Description	General	Transplantation
ACR acrolein (propenal)	the simplest unsaturated aldehyde, named and characterized in 1839, electrophilic, reactive and toxic, contact herbicide to weeds, present in tobacco smoke increases the risk of cancer, produced during cyclophosphamide treatment	 547	 29
F_2-isoprostanes	prostaglandin-like compounds formed in vivo from the free radical-catalyzed peroxidation of arachidonic acid, discovered in 1990,	 623	 22
F_4-isoprostanes	prostaglandin-like compounds formed in vivo from the free radical-catalyzed peroxidation of docosahexaenoic acid, potent biological activity as anti-inflammatory mediators	 3	0
CRA crotonaldehyde	CH_3CH, representative carcinogenic aldehyde formed endogenously through lipid peroxidation, CRA is a highly reactive aldehyde and reacts with a lysine residue in the protein, reaction with CRA and lysine residue leads to the formation of numerous numbers of adducts	 74	 53
HHE 4-hydroxy-trans-2-hexenal	oxygenated α,β-unsaturated aldehyde, other coming from omega-3 fatty acids: 4-oxo-trans-2-nonenal, 4-hydroperoxy-trans-2-nonenal, and 4,5-epoxy-trans-2-decenal	 64	 11
7KC 7-ketocholesterol (7-oxocholesterol)	toxic oxysterol, produced from oxidized cholesterol, induces: NOX, pro-inflammatory cytokines and TNF-α	 162	 6

2.3. Protein Oxidation

Biomarkers of proteins peroxidation were presented in **Table 3**.

Table 3. Biomarkers of proteins peroxidation—description and research interest.

Biomarker	Description	General	Transplantation
DiBrY dibromotyrosine	product of the reaction of hypobromous acid (HOBr) from hydrogen peroxide (H_2O_2) and bromide ion (Br^-), detected by anti-dibromo-tyrosine [DiBrY], mAb (3A5) JAI-MBY-020P	 52	5 1951, 1989, 1995, 1999, 2018
DiY/DT dityrosine (bityrosine)	biphenyl compound comprising two tyrosine residues linked at carbon-3 of their benzene rings	 1014	6 2001, 2005, 2010, 2012, 2020(2)
m-Tyrosine o-Tyrosine	abnormal tyrosine isomers, derive from oxidation of the benzyl ring of the phenylalanine by hydroxyl radical, adversely affect cells and tissues	 62	4 2003, 2007, 2008, 2012
NY 3-nitrotyrosine	specific marker of attack of peroxynitrite ($ONOO^-$) upon proteins, measured by immunostaining, HPLC, and MS in human tissues	 3554	 201
protein carbonyls	measurement of protein CO groups after their derivatization with DNPH is the most widely utilized measure of protein oxidation	 9122	 325

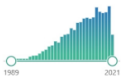
2.4. Nucleic Acid Oxidation

Oxidative DNA damage, mostly due to the hydroxyl radical, generates a huge range of base and sugar modification products [3]. They can be measured by HPLC, gas chromatography–mass spectrometry (GC–MS), liquid

chromatography–mass spectrometry (LC–MS), and antibody-based techniques, but none of these have been established as a gold standard [1]. It is estimated that each cell under normal conditions is the target of several thousand attacks on its DNA every day. Free radicals attacking DNA purines, pyrimidines, and deoxyribose create initial products, which undergo further transformations. Damage to DNA by free radicals occurs much less frequently than oxygen damage to proteins and lipids. However, the consequences are more serious due to mutagenic or immunogenic changes. One of the major products of DNA oxidation is 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 8-oxoguanine (8-oxo-Gua) [4].

Biomarkers of nucleic acids peroxidation were presented in **Table 4**.

Table 4. Biomarkers of nucleic acids peroxidation—description and research interest.

Biomarker	Description	General	Transplantation
8OHdG 8-hydroxy-2′-deoxyguanosine	**oxidized derivative of deoxyguanosine, major products of DNA oxidation, increased levels are found during carcinogenesis, increases with age, linked to the enzyme OGG1 and transcription factor NFκB**	 **5189**	 **212**
8-oxo-Gua 8-hydroxyguanine	**one of the most common DNA lesions resulting from reactive oxygen species, modifying guanine, and can result in a mismatched pairing with adenine resulting in G to T and C to A → mutation**	 **199**	**0**

3. Conclusions

Oxidative stress is a complex phenomenon resulting from the imbalance of cell homeostasis, leading to oxidative damage. It plays a vital role in the pathogenesis of many diseases. Oxidative stress is directly caused by reactive species, mainly ROS. Some of them contain unpaired electrons—free radicals, and others do not, but all of them are very reactive and cause the peroxidation of various molecules. Damage to so many cellular structures can result in the deterioration of function, including apoptosis and necrosis. Oxidative damage leaves a mark, which can be detectible by specific methodology regarding affected molecules. Those substances become the biomarkers of oxidative stress due to their ability to represent the intensity of biochemical changes accurately. The group of biomarkers, in general, comprises internal compounds related to cell physiology as well as end products of peroxidation reactions. Maintaining the cell homeostasis and redox state is a matter of competing chemical reactions and a very complex cellular and nuclear signaling mechanism. The cell can react to the environment and redistribute the energy and resources to overcome certain adverse events as oxidative stress. Oxidative damage, stress, and ROS are still intensively exploited research subjects, especially kidney disease and renal transplantation. However, the modern approach regards mainly signaling pathways and cell internal regulation; therefore, further research is necessary to translate the biochemical correlations into clinical practice and organ protection. With time, adequate interventions and solutions at the cellular level will lead to outcome improvement after organ transplantation.

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