Oxidative Stress and Overview of Pediatric Disease Biomarkers

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Abstract

Free radicals are small molecules enabled to react with other biological molecules. The antioxidant system in metabolism is responsible for balancing antioxidant levels and the amount of free radicals. Excessive free radicals production will result in oxidative stress. A certain amount of reactive oxygen species are required to maintain normal physiological activity. However, elevated oxidative stress levels will damage molecules and produce enzymatic malfunction. Several pediatric diseases are associated with increased oxidative stress. A biomarker is a specific molecule that acts as an indicator for a specific condition. There are some oxidative stress biomarkers currently in uses and further analysis of their application is required. Protein molecules may serve as potential biomarker according to proteomics analysis.

Keywords

- oxidative stress
- biomarker
- proteomics
- pediatric diseases

Introduction

Reactive Oxygen Species and Free Radicals

Free radicals can be defined as a molecule that includes one or more unpaired electron. Reactive oxygen species (ROS) are a kind of free radical associated with a single oxygen atom having a higher reactivity than O_2.[1,2] They are natural by-products of cellular function and ionizing radiation. ROS can be categorized into four groups: hydroxyl radical (OH), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$). The reactivity of these molecules is relatively low, yet they can produce hydroxyl radicals in the presence of transition metal by Fenton or Haber-Weiss reaction. Some other free radicals have biological importance as well. Lipid peroxide (ROOH), lipid peroxyl radical (ROO), and lipid alkoxyl radical are associated with lipid molecules in the cell membrane. Some species are considered as reactive nitrogen species (RNS) such as nitric oxide (NO), nitrogen dioxide (NO_2), and peroxynitrite ($ONOO^-$) whereas thiyl radical (RS) have unpaired electron.[2–4]

Oxidative Stress and Nitric Oxide System

Nitric oxide (NO) formation and oxidative stress can be classified together. NO is an uncharged, diatomic, free radical that has the ability to diffuse and affect biological molecules.[1] This molecule is produced by nitric oxide syntheses (NOSs) from L-arginine and nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) using heme, flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and tetrahydrobiopterin.[5]

NO synthases is found in the three metabolically active isoforms in mammals: Neuronal (NOS_1), inducible (NOS_2), and endothelial (NOS_3). NOS_1 and NOS_2 are soluble and NOS_3 is membrane bound. Neuronal and endothelial NO is present in various types of cells and is activated in the cell temporarily when calcium influx increases. NO binds to heme iron of soluble guanylate cyclase to produce cyclic guanosine monophosphate (cGMP) that modulates mediators such as ion channels, phosphodiesterase, and protein kinases.[6] Inducible NO, NOS_2, is activated against inflammatory and immunologic response. This isoform produces NO in the intracellular calcium state. This massive production of NO is about a 1,000 times more when compared other NO isoforms.[7]

NO can be produced by nonenzymatically and can be synthesized from nitrite at acidic pH levels under reducing conditions. Nonenzymatic NO generation may result in similar biological and enzymatically produced NO. In addition, nitrite acts as hypoxic buffer and may contribute to hypoxic vasodilation and modulation in infarction and ischemia-reperfusion tissue injury.[8]

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Proxynitrite Formation

NO can react with O_2^- and produce $ONOO^-$. Similarly, superoxide dismutase (SOD) can react with O_2^- as an antioxidant. NO reacts with O_2^- at a three times higher rate than SOD activity. The calculated diffusion rate is $6.7 \times 10^9\ M^{-1}s^{-1}$for the NO reaction whereas SOD activity is $2 \times 10^9\ M^{-1}s^{-1}$. A competition exists between NO and SOD in capturing O_2^-. Under the normal physiologic conditions, the amounts of NO and O_2^- are in much more lower concentrations when compared with SOD amount. Therefore, a limited amount of $ONOO^-$ is generated in normal conditions. In pathologic states NO and O_2^- increase in the setting of low SOD activity, resulting in toxic levels $ONOO^-$.[9]

Antioxidants

ROS has an important function in cell signaling mechanisms, especially in autocrine and paracrine systems as a defense against microbial and tumor cells.[1,2] Excess amounts of ROS may contribute to disease states or inflammation. The amount of ROS is restricted by several systems such as electron transfer, enzymatic removal, and ROS scavenging. Under normal physiologic states, the balance between ROS generation and ROS elimination is maintained by antioxidant enzymes and nonenzymatic antioxidants.[10]

Antioxidants in biological systems can be classified into three groups: enzymes, proteins, and low-molecular-weight proteins. Antioxidant enzymes are SOD, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase (GST), thioredoxin reductase, and hemeoxygenase. Proteins involved in antioxidant mechanisms include albumin, ferritin, transferrin, lactoferrin, ceruloplasmin, and thioredoxin (TRX). Other low-molecular-weight antioxidant molecules include bilirubin, tocopherols, carotenoids, ubiquinol/ubiquinone, ascorbate, glutathione, cysteine, and urate. An imbalance between antioxidant system and free radicals metabolism results in disequilibrium and causes oxidative stress.[1,2,11,12]

It is well known that high ROS and oxidative stress are correlated with some pathological conditions such as male infertility, atherosclerosis, hypertension, renal failure, neurodegeneration, carcinogenesis, and other inflammatory and degenerative conditions.[13]

Oxidative Stress Biomarkers

Biological molecules are prone to damage by reactive oxygen species. Oxidative stress may damage carbohydrates, lipids, proteins, and nucleic acids.[14] ROS are small molecules with a short half-live that requires special techniques for in vivo detection biomarkers because oxidative would not only be useful to detect oxidative damage but would also help determine the source of oxidative stress.[3,15,16] A specific biomarker would be important to determine byproduct of oxidation and to prevent further destructive effect of oxidative stress.

Oxidative stress biomarkers can be generally classified into two groups:

1. Formation of modified molecules by the effect of ROS
2. Measurement of enzymes or antioxidants

Biomarkers from body fluids such as blood, urine, semen, or cerebrospinal fluid[17] would allow the monitoring of oxidative stress in vivo that cannot be done with other invasive tests. A test that could visualize biomarkers by using a fluorescent probe would be useful, but it is not practical as a routine laboratory test.[2,8]

The first group of biomarkers includes ROS-generated byproducts that affect other biologically important molecules. This interaction can be mediated by scission, cross-linking, or covalent binding between free radicals and biological molecules. Some byproducts can be repaired or removed, but the others can stay longer if the molecule is the part of an intra- or extracellular compartment. ROS mainly attacks carbohydrates, lipids, proteins, and nucleic acids.[18] The effect of ROS on these molecules can be measured by analyzing stable byproducts produced by them. Malondialdehyde-lysine, 4-hydroxy-2-nonenal-lysine, acrolein-lysine, 8-hydroxy-2-deoxyguanosine (8-OHdG), carboxymethyl-lysine, pentosidine, and nitrite/nitrate are some clinically applicable biomarker for diagnosis of oxidative stress.[19]

Better biomarkers are necessary to improve diagnosis, targeted therapy, and therapeutic response to overcome the effect of oxidative stress. A proteomics approach based on mass spectrometry may serve as a potential protein biomarker for a clinical sample. New discoveries in proteomics methodology may allow for predicting clinically useful biomarkers with further advances in qualification, verification, assay optimization, validation, and commercialism.[20] Discovery of protein biomarker for oxidative stress may serve as a potential biomarker for clinical tests.

Studies including children are needed to investigate role of oxidative stress in pediatric disease. It could provide a better approach to understanding the effect of oxidative stress in pediatric diseases when compared with adolescents. However, a limited number of studies have reported on oxidative stress in similar children.[21,22] In most studies, oxidative stress parameters were identified in blood samples that allow glutathione peroxidase and glutathione reductase activity,[23] antioxidant vitamins,[24] uniquinol/ubiquinone,[25] and SOD and catalase activities.[26] Schock and colleagues reported oxidative stress biomarkers including antioxidant vitamins in 83 healthy children.[21] Kaufmann and colleagues identified urinary levels of F2-isoprostane in 342 children with less than 7 years population.[24] In conclusion, further studies are needed to determine reference values of oxidative stress in pediatric urine samples.

Oxidative Stress in Pediatric Diseases

Oxidative stress is associated with the progression and pathogenesis of many diseases.[6,27–30] Certain drugs such as analgesic, anticancer drugs may also contribute tissue damage by increasing oxidative stress.[31–33] Oxidative stress biomarkers have been measured in cerebrospinal fluid, joint fluid,[34] nasal lavage fluid,[35] several types of tissues,[11,12,36–38] or combination with blood or urine samples.[39–41]

Oxidative stress levels were conventionally measured by some analytical techniques including high-performance liquid chromatography (HPLC) and gas chromatography–mass

spectrometry (GC-MS).[42–44] Recent developments in monoclonal antibody technology allows for specific biomarkers via enzyme-linked immuno assay (ELISA) or Western blot analysis.[3,42] ELISA and Western blot provides a strong biomarker candidate as other analytical tests labor intensive and costly.[28,45,46] Recently, proteins were studied as potential biomarker to detect effect of oxidative stress on metabolism.[47–49] This technique needs to be improved to be useful in clinical medicine to provide rapid results. The majority of oxidative stress parameters in different pathologic states are performed by high-throughput ELISA techniques.[45,46,50]

A respiratory burst is an oxidative burst that results in the rapid production of reactive oxygen species, including superoxide and hydrogen peroxide. NADPH oxidase is an enzyme that has an ability to produce highly reactive free radicals. It is particularly involved in vascular disease and spontaneous recombination with other molecules leading to free radical production. A systematic cascade initiates the reaction of superoxide molecules with NO. This reaction results in the formation of peroxynitrite and reduces necessary bioactive NO. In addition, the superoxide anion peroxynitrite and other reactive oxygen species cause some pathology as a result of oxidation of protein and lipids. Free radicals affect redox signaling pathways and posttranslational modifications.[51] Many studies have reported a correlation between infection and the respiratory burst effect on biologically important molecules. Cemek and colleagues identified an oxidative burst in children with hepatitis A.[52] Caksen and colleagues identified lipid peroxidation and antioxidant status in children with tonsillitis.[53] Bayiroğlu monitored increased lipid peroxidation and antioxidant status in pediatric gastroenteritis patients.[54]

Proteins may serve as better potential biomarkers when compared with genome.[55] Proteomics is an evolving field in the evaluation of diseases. In addition to protein identification, quantitative proteomics provides information about the physiologic and pathologic function of identified proteins as well as their cellular localization and biological processes.[56] Differential expression of selected proteins may be used as a biomarker as a noninvasive diagnostic tool.[47,57,58] Understanding the protein expression level may be the key to understanding the cellular processes and/or pathways of a disease.[59]

Oxidative stress has destructive effect on several diseases by decomposing of biologically important molecules. Carbohydrates, lipids, nucleic acids, and proteins are prone to be denatured by the effect of free radicals and cause a loss of function. Specific biomarkers are needed to improve noninvasive diagnostic tools and develop better treatments. Recent developments have shown that proteins are potential molecules with the specificity detect qualitatively and quantitatively oxidation. Differential expression of candidate proteins can be determined by proteomic tools and quantitative proteomics may help find that target protein.

Conclusion

The challenge for further analysis in pediatric disease is elucidating molecular mechanism behind disorders and engenders the oxidative stress in mechanisms. Before accepting antioxidant therapy in clinical practice, comprehensive studies must be conducted to evaluate oxidative stress biomarkers with together clinical endpoints of patients.

This review provided updated information about noninvasive value of evaluating oxidative stress in pediatric medicine. Many of the mentioned biomarkers can be measured in urine samples where protein extraction will be needed for proteomics analysis. Measurement of these parameters has great potential for managing and treating oxidative stress-related pediatric diseases.

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