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Invited critical review

Urinary biomarkers of oxidative status

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ABSTRACT

Oxidative damage produced by reactive oxygen species (ROS) has been implicated in the etiology and pathology of many health conditions, including a large number of chronic diseases. Urinary biomarkers of oxidative status present a great opportunity to study redox balance in human populations. With urinary biomarkers, specimen collection is non-invasive and the organic/metal content is low, which minimizes the artifactual formation of oxidative damage to molecules in specimens. Also, urinary levels of the biomarkers present intergraded indices of redox balance over a longer period of time compared to blood levels. This review summarizes the criteria for evaluation of biomarkers applicable to epidemiological studies and evaluation of several classes of biomarkers that are formed non-enzymatically: oxidative damage to lipids, proteins, DNA, and allantoin, an oxidative product of uric acid. The review considers formation, metabolism, and exertion of each biomarker, available data on validation in animal and clinical models of oxidative stress, analytical approaches, and their intra- and inter-individual variation. The recommended biomarkers for monitoring oxidative status over time are F_2-isoprostanes and 8-oxodG. For inter-individual comparisons, F_2-isoprostanes are recommended, whereas urinary 8-oxodG levels may be confounded by differences in the DNA repair capacity. Promising urinary biomarkers include allantoin, acrolein-lysine, and dityrosine.

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1. Introduction

The focus of this review is to evaluate the applicability of existing biomarkers of oxidative status to human studies or epidemiological research. This involves consideration of many factors (which are discussed later, Table 1); and therefore, such evaluation can only be conducted for already studied, as opposed to novel, biomarkers. For this reason, we focus on oxygen-derived damage to biological molecules, because biomarkers of damage produced by reactive nitrogen species are less studied.

1.1. Conceptual framework: oxidative stress versus oxidative status

Reactive oxygen species (ROS) are constantly produced in aerobic organisms by normal metabolic processes, such as cellular respiration,

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Table 1
Required characteristics of biomarkers for epidemiological research.

Important considerations	Required characteristics
Relevance to biology of free radicals	1. The biomarker should be a specific product of ROS-induced oxidation
	2. The biomarker should increase in response to a known oxidative stressor (validation criterion)
Analytical issues	3. An assay for detection of the biomarker should be specific and not interfere with other substances
	4. The biomarker should be a chemically stable compound
Specimen collection and storage	5. Specimen collection should be non-invasive
	6. Storage of specimens should not produce artifactual increase of the biomarker
Application to human studies	7. The biomarker should be detectable in biological fluids of healthy individuals before the onset of a disease
	8. Measurements should not be confounded by diet or by the concentration of non-oxidized parent molecule
	9. Levels of the biomarker should have low within-person variability

antibacterial defense, and others [1]. In addition, external exposures (such as ionizing radiation, smoking, and toxins) also induce production of ROS [1]. As a result, exposure to ROS is ubiquitous, and a certain level of oxidative damage is always present in any individual. To counteract their damaging effects, aerobic organisms have developed multiple defense systems [1]. These antioxidant agents include enzymes (such as superoxide dismutase, catalase, glutathione peroxidases), sequesters of metal ions, and endogenous antioxidants (*e.g.* glutathione, ubiquinol, bilirubin, uric acid, α-tocopherol, and ascorbic acid). The two opposing processes – ROS production and antioxidant defense – set constitutive levels of ROS within the tissues and at the systemic level. Differences in both the intensity of ROS generation and the effectiveness of the antioxidant defense produce variability in oxidative status between individuals [2]. Variability in oxidative status within an individual between tissues as well as between individuals results from a complex interaction of multiple factors, including genetic [3,4] and epigenetic differences, endogenous promoters of ROS (such as iron and copper) [1], chronic inflammation [5,6] or other chronic conditions. It should be noted that most chronic conditions occur at the tissue level, while most biomarkers consider oxidative stress at the systemic level, with the implicit assumption that greater tissue-specific ROS production will be reflected by an increased systemic oxidative status. Although there is no evidence to support or refute this assumption, a distinction between tissue-specific and systemic oxidative status should be acknowledged.

The term "oxidative stress" is widely used, but, as noted by Halliwell, this term "is vaguely defined", referring to "a serious imbalance between production of reactive species and antioxidant defense" [7]. Because some levels of oxidative damage are present in every individual, the question arises as to which levels represent a "normal" (non-stress) range versus pathological elevation, which could be defined as oxidative stress. Because of this uncertainty, we believe that the term "oxidative status" is a term that can be more logically and consistently applied to both stress and non-stress states of oxidative load. For example, relatively large scale human studies ($n \geq 100$) reveal a wide variation of any oxidative status biomarker in human populations. For example, in 2828 subjects of the Framingham Heart Study, urinary levels of iPF 2α-III (a marker of lipid peroxidation) ranged from 10 to 1845 ng/mmol creatinine [8]. In 100 healthy children and adolescents, the range 8-OHdG (a marker of DNA oxidative damage) levels in urine was 4.6–27.2 ng/mg creatinine [9]. It is not clear which levels should be considered "normal" (non-stress) and which represent a serious imbalance between ROS generation and antioxidant defense (stress). The term "oxidative status" therefore seems more applicable.

1.2. Requirements for oxidative status biomarkers applicable to human studies

Because ROS have short lifetimes and cannot be directly detected in humans [10], a reasonable alternative approach is the measurement of biomarkers that are the products of non-enzymatic reactions between biological molecules and ROS [1,7]. The involvement of enzymes in the formation of biomarkers would introduce an inaccessible level of variability, and so these products do not make good biomarker candidates. Assessment of non-enzymatically formed biomarkers circumvents this problem and provides a direct index of the extent of oxidative modifications produced by ROS. Although the levels of such oxidative modifications do not measure the ROS levels *per se*, they are assumed to be proportional to the ROS levels. Therefore, the core requirement for a biomarker of oxidative status is its validation *in vivo* against a known oxidative stressor, *i.e.* a compound that produces ROS in biological systems as measured by electron spin resonance spectroscopy directly. In response to this well-recognized need, the National Institute of Environmental Health Sciences (NIEHS) has established an initiative to conduct a comparative study of biomarkers of oxidative stress (BOSS). The BOSS project tests responsiveness and specificity of the commonly used oxidative indices in an established model of oxidative stress — carbon tetrachloride (CCl_4) poisoning in rodents [11–14]. Similar to this approach, we developed a clinical model of oxidative stress, based on doxorubicin (DOX)-based chemotherapy [15,16]. DOX has been demonstrated to generate superoxide and hydrogen peroxide *in vitro*; this ROS production has been observed in animals, at pharmacological levels, using electron spin resonance spectroscopy [17,18]. This and other important characteristics for evaluation of biomarkers are presented in Table 1. Currently, only a handful of oxidative status biomarkers have been validated in either animal or clinical models.

This review focuses on urinary biomarkers because they represent the least invasive way to assess individual oxidative status and can be used in large-scale human studies. Also, urine is a better matrix than blood/plasma for measurement of oxidative modifications of biological molecules, because it has a much lower organic as well as inorganic metal content, *i.e.* lower levels of the material that can be oxidized as well as lower levels of the ROS promoters. Therefore, urine is less liable for artificial increase of oxidative markers during sample collection and storage.

2. Oxidative modifications of lipids in urine

2.1. F_2-isoprostanes

F_2-isoprostanes are formed during non-enzymatic oxidation of arachidonic acid by different types of free radicals, including reactive oxygen species [19,20]. Depending on the position where the oxygen molecule is added to arachidonic acid, four regioisomers are formed, giving each of the four F_2-isoprostane series. Furthermore, each series comprises 16 stereoisomers. Mainly two nomenclatures are used for isoprostanes (Taber et al. [21] and Rokach et al. [22]). However, other nomenclatures of isoprostanes may be found in the literature, potentially confusing readers [23].

F_2-isoprostanes can be measured in detectable quantities in human blood and urine in the general population as well as in pathological conditions [2,8,23,24]. F_2-isoprostanes and their metabolites, excreted in urine, are chemically stable compounds [25,26] and their urinary excretion levels are not sensitive to dietary intake of lipids [27–29]. The existing data indicate that levels of urinary F_2-isoprostanes are relatively stable within individuals (especially when assayed in first morning urine void) [30,31] but are widely variable in human populations [8,32], and are therefore, highly useful as biomarkers for human studies. Urinary F_2-isoprostane levels have been validated as sensitive biomarkers of oxidative stress in animal [12] and clinical