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Review Article

Urinary 8-oxo-7,8-dihydro-2′-deoxyguanosine as a biomarker in type 2 diabetes

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ABSTRACT

The increasing prevalence of diabetes together with the associated morbidity and mortality calls for additional preventive and therapeutic strategies. New biomarkers that can be used in therapy control and risk stratification as alternatives to current methods are needed and can facilitate a more individualized and sufficient treatment of diabetes. Evidence derived from both epidemiological and mechanistic studies suggests that oxidative stress has an important role in mediating the pathologies of diabetic complications. A marker of intracellular oxidative stress that potentially could be used as a valuable biomarker in diabetes is the DNA oxidation marker 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), which can be assessed noninvasively in the urine, with minimal discomfort for the patient. In this review the analytical validity of 8-oxodG is addressed by highlighting important methodological issues. The available epidemiological evidence regarding urinary 8-oxodG and type 2 diabetes is presented. A possible role for DNA oxidation in cancer development in type 2 diabetes patients is discussed, followed by an evaluation of the potential of urinary 8-oxodG as a clinical biomarker in type 2 diabetes.

Contents

Introduction

Diabetes mellitus constitutes a major global health problem because of its increasing prevalence and the accompanying risk of serious complications. According to recent estimates from the International Diabetes Federation 285 million people worldwide have diabetes, representing 6.6% of the world population, and it is

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predicted that this figure will increase to 438 million in 2030 [1]. In most countries diabetes is one of the major causes of premature illness and death, and the associated cardiovascular disease causes the death of 50% or more of diabetes patients [1].

The huge morbidity and mortality associated with diabetes together with the increasing prevalence calls for additional preventive and therapeutic strategies. New biomarkers that can be used in therapy control and risk stratification as alternatives to current methods, e.g., HbA1c and urinary albumin excretion, are needed and can facilitate a more individualized and sufficient treatment of diabetes.

In diabetes management the currently available biomarkers are of extracellular origin and are most of all "by-products" of the disease that do not provide information on the intracellular environment under diabetic conditions. The identification of new biomarkers that actually reflect the intracellular pathological processes could improve diabetes care.

Over the past decade there has been an increasing focus on the role of oxidative stress in the pathophysiology of diabetes-related complications. The diabetic state is associated with increased levels of markers of oxidative stress and evidence derived from mechanistic studies suggests that oxidative stress has an important role in mediating the pathologies of diabetic complications [2–5]. A marker of intracellular oxidative stress that potentially could be used as a new biomarker in diabetes is the DNA oxidation marker 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG). This marker can be assessed noninvasively in the urine, with minimal discomfort for the patient, which makes it suitable for use in risk stratification and therapy control.

8-OxodG is a frequently measured urinary marker of oxidative stress, and in this review we discuss the potential role of urinary 8-oxodG as a biomarker in diabetes. Some 90% of diabetic individuals have type 2 diabetes mellitus, and consequently type 2 diabetes represents the focus of our discussion.

Oxidative stress and type 2 diabetes

Despite considerable research, the exact cellular and molecular mechanisms responsible for type 2 diabetes and its complications are still unresolved.

Oxidative stress is defined as an imbalance between production of reactive oxygen and a biological system's ability to detoxify the reactive products or repair the resulting damage. Disturbances in the normal redox state can cause toxic effects through generation of reactive oxygen species (ROS) that can damage all components of the cell, including lipids, proteins, and nucleic acids (nucleic acid oxidation) [6].

ROS are produced in various tissues under diabetic conditions and are possibly involved in both the progression of pancreatic β-cell dysfunction and the insulin resistance found in type 2 diabetes. In addition, ROS seem to play an important role in the progression of atherosclerosis seen in diabetes patients [7].

DNA oxidation

ROS can cause the formation of a large number of pyrimidine- and purine-derived lesions in DNA, and 8-hydroxylation of guanine (8-oxoGua or 8-OHGua) is one of the most widely studied lesions. Oxidized nuclear DNA undergoes repair and the repair products 8-oxoGua and its corresponding deoxyribonucleoside equivalent 8-oxodG are excreted into the urine [8]. Although it is currently unclear exactly which repair process is the most important, observations point to DNA repair as the predominant process in producing urinary 8-oxodG, with only a negligible contribution from cell turnover and diet, and thus the measurement of 8-oxodG, rather than 8-oxoGua, is preferred as a biomarker of oxidative damage to DNA [9].

The contribution from the deoxyribonucleotide pool to the total amount of excreted 8-oxodG remains to be determined but seems to be negligible owing to the small pool size compared to total DNA [10,11].

In the steady state the amount of excreted 8-oxodG will equal the newly formed 8-oxodG, and the urinary excretion of 8-oxodG will be equal to the rate of oxidative damage to DNA. Hence, urinary excretion of 8-oxodG reflects the average rate of oxidative damage to DNA in the whole body [12].

Many different cell types have shown increased levels of 8-oxodG in the diabetic state in both human and animal models, which indicates that there is a generalized increased level of oxidative stress in diabetes. The majority the evidence regarding tissue levels of 8-oxodG is derived from animal studies. In rat models of streptozotocin-induced diabetes 8-oxodG has been shown to be higher in several tissues of the diabetic rats, including kidney, liver, heart, retina, and nerve tissue, compared with nondiabetic controls [13–17]. Studies of human cells have shown increased levels of 8-oxodG in the vitreous of patients with diabetic retinopathy [18], in islets of pancreatic tissues of type 2 diabetes patients [19], and in isolated human endothelial cells exposed to high glucose [20].

Methods for measurement of urinary 8-oxodG

The two major analytical approaches that are used for the measurement of urinary 8-oxodG are chromatography, combined with electrochemical detection or mass spectrometry, or direct immunodetection. A few interlaboratory comparisons between some of the chromatography-based techniques and enzyme-linked immunosorbent assay (ELISA) have been made for the quantification of 8-oxodG in urine [21–23]. They showed a reasonable agreement between the chromatography-based techniques, but the ELISA-based techniques gave higher values and showed more variability. Lack of specificity seems to be the main issue in the ELISA method, which presumably is because the epitope (8-hydroxylation in the 8-position) resembles many molecules that are present in biological samples. The greater variability of the ELISA method and measurement of background levels of additional compounds that are recognized by the antibody can reduce the assay's ability to detect differences.

Among the sufficiently sensitive methods to measure urinary 8-oxodG only liquid chromatography with mass spectrometry that includes qualifier and quantifier ion detection [24] and gas chromatography coupled to mass spectrometry meet the recommended requirements for identification and quantification set by the Commission of the European Communities [25].

When determining the urinary excretion of 8-oxodG, 24-h urine collection is preferred. Because 24-h collection can be cumbersome, often resulting in incomplete or improper collection, spot urine samples are frequently used instead. Urinary creatinine concentration is used to adjust for the varying dilution of these spot urine samples, but the interpretation of the results is often difficult because creatinine in itself is closely associated with other important variables such as sex, age, body mass index, and race. In large cohort studies 24-h collection is often not practically possible, and in these situations creatinine-corrected spot urine samples can be used, if the uncertainties associated with this correction are handled appropriately. One way of doing this is to assess the effects of 8-oxodG and creatinine separately in multiple regression models as described by Barr et al. [26]. Although 24-h urine is considered the gold standard in paired and randomized trials, bias from creatinine correction is less likely. In these designs spot urine with creatinine correction can be used without considerable risk of bias.

Urinary 8-oxodG as a marker of oxidative stress in type 2 diabetes

Studies of the associations between urinary 8-oxodG and diabetes or diabetes-related quantitative traits are presented in Table 1.