The Potential Use of Glutathionyl Hemoglobin as a Clinical Marker of Oxidative Stress

Over the past decade, there has been substantial interest in oxidative stress and its potential role in the development of disease-related pathophysiological complications in diabetes (1, 2), atherosclerosis and associated cardiovascular disease (3, 4), cancer (5), aging (6), and other conditions. “Oxidative stress” refers to an imbalance between antioxidant and oxidant-generating systems. An increase in oxidative stress can have a profound effect on lipoprotein modification, transcription, and cell function and metabolism. Oxidative stress can arise via various mechanisms associated with excessive oxygen radical production, such as autoxidation of glucose and glycated proteins, and glycation of antioxidant enzymes. Even in healthy subjects, hyperglycemia, increased free fatty acids, and hyperinsulinemia can trigger oxidative stress (7).

In diabetes, increased blood concentrations of markers of oxidative stress, especially in patients with poor glycemic control (8, 9), have been implicated in the development of vascular complications. The finding of increased oxidative stress in newly diagnosed children and diabetic patients with no complications (8) suggests that the increased oxidative stress in diabetes may not be attributable to the complications but could contribute to the development of complications. Other studies in patients with increased concentrations of plasma lipoproteins (10) have implicated increased oxidative stress with associated oxidation of lipoproteins (11, 12) as playing a role in increasing atherogenic risk and associated cardiovascular disease (13, 14).

The finding of increased oxidative stress in conditions such as diabetes and hyperlipidemia has suggested the use of antioxidant supplementation as a potentially useful therapy in preventing or delaying the development of complications such as atherosclerosis and heart disease. Consequently, the availability of clinical markers that can provide an accurate assessment of the degree of oxidative stress will become important in clinical trials aimed at investigating the effectiveness of antioxidant therapy for preventing or reducing the risks of complications in diseases such as diabetes and hyperlipidemia.

In the last issue of Clinical Chemistry, Niwa et al. (15) examined the possibility that glutathionyl hemoglobin (16) is a serum marker for oxidative stress. Using liquid chromatography/electrospray ionization-mass spectrometry, they found that glutathionyl hemoglobin was significantly increased in type 2 diabetic patients and patients with hyperlipidemia. They suggested that this reflected increased oxidative stress because incubation of hemoglobin and reduced glutathione with hydrogen peroxide led to an increase in glutathionyl hemoglobin. Reduced glutathione protects against free radical injury by eliminating reactive oxygen species and inhibiting oxidation of protein thiol groups.

Niwa et al. (15) present a potentially novel and accurate assay for serum oxidative stress, which raises interesting questions. Does their assay provide a clinically useful marker of oxidative stress? Do the results provide a meaningful assessment of risk for development of pathophysiology associated with increased reactive oxygen species in diseases such as diabetes or atherosclerosis? Rigorous clinical trials must assess patient populations representing a broad spectrum of complications and with a diversity of conditions (17) that contribute to increased oxidative stress. Will hemoglobinopathies or other hematological abnormalities modify hemoglobin molecules and/or concentrations of hemoglobin (18, 19) and confound the interpretation of the assay results? These measurements will also need to be compared with currently accepted markers of oxidative stress (20), such as measurements of the lag phase of copper-catalyzed LDL oxidation, F2 isoprostanes (10), lipid oxidation (malondialdehyde), protein glycation, glycosidation products (pentosidine and carboxymethyllysine), and methylglyoxal (21). Additionally, this assay must be compared with newly evolving assays of tyrosine nitration and protein carbonyl formation as early markers of tissue oxidative damage (22, 23). The technology used for the present assay of glutathionyl hemoglobin will not be easily available, but if this assay proves to be a reliable measure of oxidative stress, then glutathionyl hemoglobin concentrations could be used as a bench mark for similar assays. Electrospray ionization mass spectrometry has been proposed for use in standardization of hemoglobin A1c measurements (24).

In diabetes, increased oxidative stress has been implicated as playing an important role in the development of diabetic complications. To date, the accepted clinical marker for assessing long-term glycemic control in diabetic patients has been the measurement of hemoglobin A1c. The Diabetes Control and Complications Trial (25) and the United Kingdom Prospective Diabetes Study (26) showed that improved glycemic control, as measured by a reduction in hemoglobin A1c, significantly reduced the risk for development and/or progression of all diabetic complications. Furthermore, the Cambridge Heart Antioxidant Study showed a significant risk reduction for cardiovascular events (27) in patients with symptomatic coronary atherosclerosis who received vitamin E supplementation. Niwa et al. (15), however, found no correlation between hemoglobin A1c and glutathionyl hemoglobin in their study population of type 2 diabetic patients. This would suggest that increased oxidative stress was independent of glycemic control. On the other hand, other investigators have reported increased oxidative stress in patients with poor glycemic control (2). Thus, it is clear that additional studies in larger and broadly representative diabetic populations will be needed to determine the relationship between glutathionyl hemoglobin and glycemic control and whether oxidative stress, independent of glycemic control, is associated with the development of...
diabetic complications (28). Nevertheless, an accurate measurement of oxidative stress can play an important role as an outcome measure in clinical studies aimed at determining the beneficial effect of antioxidant therapy in diabetic patients (29).

In hyperlipidemia, Niwa et al. (15) did not investigate possible associations between their assay for oxidative stress and lipid concentrations. Additional studies are needed to determine whether increased glutathionyl hemoglobin as a measure of oxidative stress is related to increased lipid concentrations and whether a measure of increased oxidative stress associated with increased lipid concentrations is associated with increased atherogenesis. Again, the use of serum glutathionyl hemoglobin may provide a useful clinical marker of the effectiveness of antioxidant treatment in clinical trials investigating the efficacy of antioxidants in ameliorating pathophysiological complications associated with increased oxidative stress.

Improved diagnostic tests and improved accuracy of these tests can improve the evaluation of the effectiveness of a particular therapy. Although clinical use of rigorously evaluated tests cannot guarantee better patient outcomes, improvement in patient care can be expected when therapy can be guided by such tests. Ideally, such tests should be subjected to randomized clinical trials to determine the contribution of use of the tests to health outcomes.

References


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