
Saliva Analysis for Monitoring Dialysis and Renal Function

Measurement of biological markers that demonstrate distinguishable and regular changes from pre- to post-dialysis states can enable necessary monitoring of dialysis efficacy and the level of renal function in patients with end-stage renal disease.

In a report in this issue of *Clinical Chemistry*, Blicharz et al. (1) suggest that the measurement of biomarkers in saliva may be an effective alternative method for monitoring the effectiveness of hemodialysis. In particular, Blicharz et al. highlight as markers of interest 2 small molecules present in saliva, nitrite and uric acid (UA).¹ Monitoring of markers in saliva instead of serum is advantageous because saliva collection is a noninvasive, simple, and inexpensive approach with minimal infectious risk that can be performed by the patient with no need for involvement from medical personnel. Saliva can be tested at home, thus saving the need for a visit to the clinic or hospital.

Markers for monitoring patients with end-stage renal disease must fulfill 3 requirements: (a) the markers should properly reflect serum concentrations of toxins to be dialyzed, (b) the correlation between the serum and saliva concentrations of the markers should be as high as possible, and (c) the concentrations of the markers in saliva should not be altered by intraoral conditions or by processes associated with marker transport from serum into saliva.

Whole saliva is composed of components that originate in the major and minor salivary glands as well as from nonsalivary glandular sources, and the composition of saliva may vary under resting vs stimulated conditions (2). More than 90% of saliva is secreted by the major salivary glands, which include the parotid, submandibular, and sublingual glands, whereas only a small portion of saliva originates in minor salivary glands and intraoral sources such as oral mucosa or gingiva. Contribution to the saliva from gastrointestinal reflux is minute and of negligible importance under normal conditions. Various components of saliva are either passively diffused or actively transported directly from the serum into the saliva through the oral mucosa and/or gingiva. The composition of such components in saliva may or may not reflect their serum composition (3–5). The watery component and the electrolytes in saliva are derived from serum, but the various immunoglobulins, enzymes, and proteins may originate in the serum, the salivary glands, or other intraoral and extraoral sites.

Analysis of saliva composition may be used as a diagnostic tool for the localization and assessment of various systemic diseases (such as end-stage renal disease). Such analysis must be based on a broad understanding of the specific concentrations and origins of the various immunological and biochemical components of saliva (6, 7). The composition of saliva is affected by 2 fundamental mechanisms:

1. *The source of saliva.* The salivary glands and oral saliva sources may be the primary sites in which the various components are produced, or they may serve as sites through which various components are passively diffused or actively transported from the serum. The lack of a high correlation between concentrations of a component in saliva and in serum does not necessarily disprove the serum origin of that component but may simply reflect variability in the diffusion process for the component. When concentration correlations are high, however, the source of the specific component in the saliva can be concluded to be serum (3–5).

2. *Oral cavity modulation of saliva.* After secretion the composition of saliva is altered by various processes that take place in the oral cavity (4). Saliva composition is highly variable, much more so than serum composition. Accordingly, the reader assessing the importance of the Blicharz et al. study should not be surprised that not all of the results obtained support the trends anticipated, i.e., not all measurements in all patients demonstrated significant decreases in post- compared to predialysis saliva concentrations of nitrite and UA. Because of the physiological variability of saliva composition, multiple measurements must be performed, a process that decreases SDs and significantly improves the diagnostic sensitivity and specificity of such measurements. If circumstances allow multiple saliva collections and analyses and these are inexpensive and accurate, then the use of saliva for the monitoring of dialysis efficacy can be recommended.

Important questions to be addressed in regard to saliva monitoring are what molecules are the best biomarkers and what molecules best fulfill the 3 requirements previously specified. Of the few molecules Blicharz et al. examined, UA and nitrite best fulfilled these criteria. The authors point out that measurement of pre- and posttreatment blood urea nitrogen is the gold standard test for evaluating dialysis efficacy, but conflicting observations have been reported regarding the

correlation of saliva urea with blood urea nitrogen. Thus Blicharz et al. were seeking alternative saliva markers that might prove useful for noninvasive monitoring of dialysis efficacy and renal function. We have previously reported on 2 molecules, blood urea nitrogen and potassium, whose measurement can be valuable in assessing the general condition of almost every hospitalized patient. We found these analytes to demonstrate very high correlation coefficients between serum and saliva: $r = +0.85$ and $+0.50$, respectively. Accordingly, monitoring of urea nitrogen concentrations should certainly be considered as part of routine saliva assessment in patients undergoing dialysis. We recommend further analysis in much larger cohorts of dialysis patients to investigate the efficacy and feasibility of blood urea nitrogen analysis performed according to the method employed by Blicharz et al. The potential role of potassium and other small molecules and ions that diffuse into the saliva from the serum also should be evaluated. The 2 molecules suggested by Blicharz et al. seem to at least provide a “proof of a concept” and may be suitable candidates for saliva monitoring to assess dialysis and renal function. Both molecules are also involved in various biological processes in the oral cavity and are related to various oral pathologies such as oral cancer and other oral pathologies related to free radicals.

UA is considered the most important antioxidant molecule in saliva and contributes approximately 70% of the total antioxidant capacity of saliva. Correlations between concentrations of UA in saliva and serum indicate that UA in saliva originates in serum (6, 8, 9). UA may certainly be recommended as a biomarker for assessing dialysis because UA concentrations are profoundly decreased by dialysis (10–12). Saliva nitrites are derived from the production and metabolism of saliva nitrosamine derived from the absorption of dietary nitrates (NO_3^-) in the upper gastrointestinal tract and their active concentration from the plasma into the saliva by the salivary glands. This process occurs through an active transport system similar to that for iodide, thiocyanate, and perchlorate (13). In the oral cavity nitrates in saliva are turned into nitrites (NO_2^-), which play an important role in carcinogenesis by reacting with amines and amides to form the carcinogenic nitrosamines, thus initiating and promoting oral cancer (14, 15). Oral cancer is the sixth most common human cancer, with an increasing incidence in younger people, a high morbidity rate, and a 5-year mortality rate of about 50%. Free radicals such as reactive oxygen and nitrogen species, which induce oxidative and nitrative stress, are principal inducers of oral cancer. Reactive nitrogen species in the form of the nitrosamines NO_3 and NO_2 and reactive oxygen species such as superoxide radicals (O_2^-), hydroxyl radicals (OH^\cdot), and

hydrogen peroxide (H_2O_2) play key roles in human cancer development through base alterations and strand breaks in DNA, damage to tumor suppressor genes, and enhanced expression of protooncogenes. Protein damage associated with reactive oxygen species may also induce mutations. UA is the major antioxidant molecule in saliva that reacts with and neutralizes reactive oxygen species in the oral cavity; therefore measurement of UA concentrations along with NO_2^- , as Blicharz et al. suggest, is highly warranted, not only in patients with end-stage renal disease undergoing dialysis but also in patients with other oncological and oxidative-related disorders.

In conclusion, although much more research is needed, the report by Blicharz et al. provides early evidence of the usefulness of saliva biomarkers, particularly for monitoring dialysis. The use of saliva samples obtained with simple, inexpensive, and user-friendly test strips is a very attractive alternative to the use of blood samples and may revolutionize care-monitoring strategies for dialysis patients as well as for patients suffering from other chronic diseases.

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