Original Research

Salivary Cortisol Results Obtainable Within Minutes of Sample Collection Correspond With Traditional Immunoassays

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ABSTRACT

Purpose: Cortisol is frequently assayed as a stress-responsive biomarker which changes over the course of minutes to meet the demands of a person's social context. Salivary cortisol is often used as a noninvasive sampling method that possesses important health implications. A critical barrier to psychobiological research that involves salivary cortisol is a time delay of days to months before cortisol results are obtained via immunoassay, long after the person is no longer proximate to the social context in which they provided the sample. The present study was designed to address this critical barrier through creation of a lateral flow test (LFT) cortisol device capable of measuring salivary cortisol within minutes of sample collection. The LFT is frequently used within commercial point-of-care settings to obtain rapid answers to the presence/absence of a biomarker. The present study extends the LFT into the research domain by presenting performance characteristics of a quantitative LFT that measures salivary cortisol within 20 minutes of sample collection.

Methods: Saliva samples from 29 adults (15 men) were obtained in the morning and afternoon by using Passive Drool and then the Super · SAL Extra Collection Device (hereafter Super · SAL) and later assayed with LFT and a commercially available enzyme immunoassay.

Findings: Results indicate the LFT correlated well with these collection methods ($R = 0.872$ with Super · SAL, $R = 0.739$ with Passive Drool, $P < 0.0001$) and at comparable levels to correspondence of Super · SAL with Passive Drool ($R = 0.798$, $P < 0.0001$) which were measured with the same assay.

Implications: These results open an exciting new possibility to integrate this technologic advance into stress research, including knowing and potentially changing the person's social context in a time-sensitive manner. Methodological improvements such as this have the possibility of refining conceptual models of stress reactivity and regulation. (*Clin Ther.* 2015;37:505–514)

Key words: collection methods, cortisol, immunoassay, lateral flow technology, stress regulation.

INTRODUCTION

Stress is a leading cause of morbidity and mortality in the United States.[1] A small set of biomarkers provide information about chronic and acute stressors.[2] Cortisol is putatively the most frequently investigated stress biomarker[3] because cortisol is linked with many physiologic processes such as neural development and cell death,[4] immune function,[5] learning and memory,[6] sleep,[7] metabolism and fat distribution,[8] growth and development,[9] reproduction,[10] and aging.[11] The powerful role of cortisol in shaping health outcomes is illustrated by its clinical value in treatments that range from mild rashes in over-the-counter creams to life-saving efforts.[12]

Cortisol is the end product of the hypothalamic-pituitary-adrenal (HPA) axis. After appraisal of the

Accepted for publication February 12, 2015.
http://dx.doi.org/10.1016/j.clinthera.2015.02.014
0149-2918/$ - see front matter

social context in limbic and paralimbic neural circuitries,[13,14] the hypothalamus releases corticotropic releasing hormone that initiates a hormonal cascade that culminates with adrenocorticotropic hormone that stimulates the release of cortisol from the adrenal cortex and into the blood.[15] After ~15 minutes from the onset of a stressor, cortisol concentrations peak.[16–18] As a lipid-soluble hormone, cortisol easily crosses through cellular membranes which allows it to travel directly to cell nuclei to change gene expression,[19,20] especially in the brain where it is responsible for terminating the stress response[4] via negative feedback.[21,22] Cortisol also acts throughout the body where it influences physiology over seconds, minutes, hours, and days.[23] Salivary measurement of small steroids such as cortisol take advantage of the fact that free cortisol is lipid soluble; this biologically active fraction of total cortisol passes through the acinar cells to enter saliva via passive diffusion in proportion to cortisol's entrance into cell nuclei.[24]

Measuring cortisol in saliva has opened a window of opportunity to conduct stress-related research that involves many repeated measures[25] or applications with vulnerable populations[26,27] or in unique settings.[28,29] Salivary measurement has even indicated unique diagnostic and treatment information about cortisol-related diseases such as Addison syndrome or Cushing disease which previously required much more invasive measurements throughout treatment.[30–33] First-generation salivary cortisol assays relied on radiolabeled cortisol antibodies in radioimmunoassays that were highly sensitive and specific. This sensitivity was enhanced with the next generation of assays that quantified cortisol concentrations via optical density measurements in commercially available enzyme (EIAs) or luminescence immunoassays (LIAs) that required much lower sample volumes to be effective. These particular methods rely on the degree of color change of bound horseradish peroxidase to estimate hormone concentrations relative to a standard curve. The range of sensitivities with the use of these assays provided a limit of detection in the range of picogram per milliliter, a necessary prerequisite for salivary steroid hormone detection whereby concentrations are low.

A critical barrier in the stress field is that current immunoassay methods do not provide reportable results for cortisol for days to months after collection. Recent studies are beginning to explore the feasibility of point-of-care measurement of salivary cortisol,[34–37] typically using lateral flow test (LFT) assays to deliver qualitative or quantitive test results within minutes of sample collection.[38] The present study describes the first in a new generation of assays for measuring salivary hormones such as cortisol by using an LFT device (Oasis Diagnostic Corporation, Vancouver, WA). The first US patent that used the term *lateral flow* was filed in 1988 and was awarded in 1990,[39] although several companies had patented elements of that concept earlier. The first successful commercial use of an LFT was the EPT urine human chorionic gonadotropin hormone dipstick pregnancy test. LFT has been widely used in industry since the late 1980s and early 1990s, primarily to target the commercial needs for point-of-care assays.[40] In similar fashion to EIA and LIA, LFT relies on the detection of an emitted signal (in this case fluorescence) to provide a fully quantitative cortisol readout, but, unlike EIA and LIA or even technologies such as mass spectrometry or microarrays, LFTs are not widely used in the academic community. The reasons for this may be related to education, intellectual property considerations, and a lack of LFT suppliers that cater to the specific needs and recommendations of researchers.

The cortisol LFT technology is a unique proprietary format that improves on several related patented technologies.[41–43] We designed this LFT specifically for the research community to provide major advantages over currently available methods for saliva and include the following: (1) the assay takes minutes from sample collection to end results; (2) the assay and reading unit (Litebox Image Analysis Module, [LIAM]) are portable, allowing real-time cortisol assessment to be performed in a variety of point-of-care and nontraditional settings; and (3) the assay retains many of the advantages of available methods (eg, high sensitivity and quantitative output), so few sacrifices in assay quality are required for real-time, point-of-care cortisol measurement. The present study describes the methodology and performance characteristics of real-time LFT cortisol, including comparison of this technology with another commercially available non-LFT assay. The goal was to determine correspondence and, by extension, viability of obtaining real-time cortisol scores within the near future.

METHODS

Participants

All procedures were approved by the institutional review board at a research university, and all participants provided informed consent. Participants were excluded if