Salivary biomarkers for Huntington’s disease

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ABSTRACT

OBJECTIVE: The objective of the current study was to assess the potential for saliva to serve as a biosensor for accessible biomarkers for Huntington’s disease (HD).

METHODS: In the current study, we measured Htt protein in saliva from manifest HD patients, gene-positive premalignant HD patients, and age- and sex-matched normal individuals (controls). The human saliva volume was collected and Htt measurements were performed using ELISA methods. Additional salivary analytes, including alpha amylase, cortisol, C-reactive protein (CRP), and uric acid, were also measured using standardized ELISAs.

RESULTS: Salivary total Htt levels were significantly increased (p=0.0012) in saliva from HD individuals (mean±0.775 ng/ml) compared to normal controls (mean±0.359 ng/ml). Salivary total Htt did not vary over time of day or over different days, nor were there age or gender effects. Additionally, salivary total Htt levels were higher in gene positive premalignant HD subjects compared to normal controls (p=0.05). CRP, a widely used biomarker of systemic inflammation, was found to be significantly (p=0.025) elevated in premalignant HD subjects (9.54±5.3 mg/dL) compared to normal controls (3.39±pg/mL) and may be an early marker for disease onset.

CONCLUSIONS: Measurements of salivary Htt and other disease proteins offers significant promise as relevant, non-invasive biomarkers of disease onset and progression in HD.

INTRODUCTION

Biomarkers for HD: The mutation responsible for Huntington’s disease (HD) is an abnormal CAG repeat expansion in the HTT gene that encodes huntingtin (Htt), a large protein of 3,414 amino acids. Pathology in HD arises mainly from the expression of mutant Htt, which is linked to the formation of toxic soluble protein oligomers as well as insoluble aggregates, and contributes to the degeneration of many intracellular organelles, including the primary pathological hallmark of HD, the formation of polyglutamine inclusions also occurs in HD at specific locations. Further, patients with HD exhibit multiple peripheral changes, including skeletal muscle abnormalities and peripheral immune system dysfunction.

Measurements of Htt in the brains of patients are not possible, hence several studies have turned to measuring Htt in CSF and blood (1), from human patients in order to assess the utility of Htt to serve as a biomarker. However, CSF collection requires a lumbar puncture, which is invasive and, and CSF levels of Htt are known to be very low. Measurements of Htt in blood have been successful, however levels can vary depending on the blood cell type, large starting volumes (i.e. 50 ml) are often required and blood drawing itself is also an invasive technique. In this study, we explored whether Htt and other disease proteins could be reliably measured in saliva from HD patients and normal individuals.

Saliva as a biospecimen: Saliva is a composite of oral fluids secreted from many different glands, including the major and minor salivary glands, as well as the buccal glands, saliva contains a wealth of hormones, proteins and nucleic acid molecules that reflect physiological status. Importantly, several disease proteins have been identified in human saliva, such as amyloid beta (2-1), and tau (3), which are associated with Alzheimer’s disease, and alpha-synuclein (4) and DJ-1 (5), which are associated with Parkinson’s disease. Salivary levels of these proteins are thought to represent useful biomarkers for these diseases. In this study, we report that significant and reproducible levels of Htt can be detected in saliva from human subjects and that salivary Htt is correlated with HD symptoms, suggesting utility as a biomarker for symptom onset and, perhaps disease severity.

METHODS

Participants: Patients were recruited through the UCSD HDSA Center of Excellence under an IRB-approved protocol. Marked HD inclusion criteria included a definitive diagnosis of HD with expanded trinucleotide CAG repeat of ≥36. Controls have no history of neurological or psychiatric disorders, and no use of psychoactive substances or medications.

Sample collection: All donors refrained from smoking, eating, drinking, or oral hygiene procedures for at least 1 hr prior to sample collection; then rinsed their mouths thoroughly with water. Samples were collected between 9 am and 12 noon using the passive drool method. At the time of use, salivary samples were thawed and centrifuged at 10,000 rpm for 10 mins to remove insoluble material and cell debris. Supernatants were collected for all assays. Total protein in the supernatants was determined using the BCA protein assay kit (Pierce).

Western Blot: Human saliva supernatants were concentrated 4-fold by vacuum centrifugation or used as un-concentrated “Tear”. Samples were separated by 7% SDS-PAGE and blotted onto nitrocellulose membranes using standard techniques. For immunofluorescence, the Htt antibody MB1216 (Millipore) was used at 1:400.

ELISA: Total protein levels in salivary samples were quantified using certified ELISA kits from LifeSpan Biosciences, Inc. (Salt Lake City, UT) following the manufacturers protocol. The recombinant protein standard included in this kit corresponds to the non-phosphorylated form of the human Htt protein, with antibodies corresponding to proteins including this region. Samples were assayed for total Htt using the ELISA kit according to the manufacturer’s instructions. The recombinant protein standard in this kit corresponds to the non-phosphorylated form of the human Htt protein, with antibodies corresponding to proteins including this region. Samples were assayed for total Htt content with a BCA protein assay kit (Bio-Rad, Inc.).

Htt levels in salivary were measured using ELISA kit for total Htt (LSBio), following directions by the manufacturer. Differences in Htt levels were determined by ANOVA followed by post-test comparing all groups to the normal control (Students t-test). Bar graph on right shows the difference between all gene (+) individuals and normal controls. Further, the correlation coefficient was determined using One-way ANOVA followed by Dunnett’s post-test comparing all groups to the normal control group. Linear regression analysis (Spearman correlation) was used to compare Htt and other disease protein levels with clinical variables, as these measures were not normally distributed. Differences between males and females were determined using Student’s t test (unpaired, two-tailed). All analyses were performed using GraphPad software (Phoenix).

SUMMARY

• Full-length Htt and other disease proteins can be detected in human saliva.
• Total Htt levels are elevated in saliva from HD patients; mHtt levels are elevated in pre-symptomatic patients (data not shown). These could represent indicators of disease symptoms and markers for Htt-lowering therapeutics.
• Salivary Htt levels did not vary with gender, age, time of day, or on different days.
• Salivary Htt showed a significant positive correlation with the UHDRS TFC score.
• Non-affixed family members showed elevated salivary cortisol, possibly indicative of an abnormal stress response.
• CRP levels are elevated in pre-symptomatic patients, possibly suggestive of a pathologic inflammatory or metabolic state in HD and an early marker for disease.

CONCLUSIONS

• Measurements of salivary Htt and possibly other salivary proteins, offer significant promise as relevant, non-invasive biomarkers of disease onset and severity in HD.