**Serum and Saliva Analysis by Differential Scanning Calorimetry as a Novel Diagnostic Modality in Head and Neck Cancer: A Pilot Study**

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**ABSTRACT**

Objective: To evaluate the serum of patients with head and neck cancer for trends in thermogram signature through differential scanning calorimetry (DSC). Additionally, to determine the feasibility of salivary sample DSC processing and interpretation.

Methods: Twenty one patients with a new diagnosis of oral or oropharyngeal squamous cell carcinoma and 20 healthy control patients were identified for participation. Each patient provided a serum and saliva sample for DSC testing along with a pertinent medical history questionnaire. Serum samples were tested through DSC, producing a unique thermogram signature for each patient. Using a novel technique, salivary samples were filtered and buffered to standardize them for DSC processing. Each salivary sample successfully produced a unique thermogram signature. Thermograms were sorted based upon tumor location and patient demographics, individually analyzed, interpreted against healthy controls.

Results: Each of the samples successfully produced a unique thermogram signature. When serum thermograms from the test population were evaluated based upon tumor location, clear deviations in peak excess specific heat capacity from healthy controls were observed between 60 and 75 degrees C. These results were observed in all tumor locations with the exception of tonsillar disease, which showed minimal to no deviation from normal samples. Tumor T-stage was also found to significantly alter thermogram patterns in a predictable manner.

The novel salivary sample processing protocol proved to be successful and reproducible. However, significant variability was observed in salivary sample thermograms despite this standard collection and processing protocol. The trends identified in serum sample thermograms based upon tumor location and stage were not clearly evident in salivary thermogram interpretation.

Conclusions: DSC has proven to be a valid addition to the proteomic investigational armamentarium. Through interactions with human serum albumin, minute changes in low molecular weight protein expression are detectable through serum thermogram interpretation. Evaluation of a large patient population is necessary to further evaluate this technology for use as a diagnostic screening test for head and neck carcinoma. Due to the sensitivity of this technology, a more stringent collection protocol may be necessary to more precisely evaluate salivary thermogram trends.

**BACKGROUND**

Head and neck cancer comprises more than 5% of all cancer diagnoses in the United States, and an even higher percentage worldwide. Despite this high incidence, little change has been seen in survival over the last 30 years. Many of these tumors present in advanced stages due to the difficulty in early diagnosis that accompanies the inaccessibility of these lesions. Unlike many other common forms of carcinoma, there are currently no accepted screening modalities in place to assist with the early diagnosis of head and neck tumors.

Differential scanning calorimetry (DSC) has gained recent recognition in the analysis of human serum as a detection mechanism for changes in systemic protein expression. The technology measures the energy released from the proteins that comprise a given sample as they uncouple under controlled changes in temperature. The result is a thermogram ‘signature’ displaying energy released from a sample as a function of heat applied. Samples obtained from healthy controls can be contrasted against individuals with known disease processes to identify changes in protein expression through changes in the thermograms produced.

The ten most abundant proteins in human serum account for nearly 90% of the serum by weight, led by albumin, IgG, fibrinogen, and haptoglobin. The next 9% is accounted for by twelve proteins, and nearly 3000 proteins account for the final 1%. It is this final 1% that contains the differential protein expression found within disease states. Interestingly, the thermogram does not possess the detail to display them individually. Rather, it is their ability to bind to and alter the denaturation temperatures of the most abundant proteins in the serum that accounts for the significant changes seen in the thermograms produced in various disease states.

The protein composition of human saliva has been well delineated through gel electrophoresis and mass spectroscopy, but has it not been previously evaluated by DSC. While changes in salivary protein expression are seen in head and neck cancers, the effects on protein interactions, and thus thermogram signatures, have yet to be defined.

**OBJECTIVE**

1. Assess differential scanning calorimetry as a screening and diagnostic tool for cases of head and neck carcinoma.
2. Identify trends in thermogram signature that correlate with staging or prognostic significance
3. Assess the feasibility of human saliva as a new medium for DSC, and any trends identified in sample obtained from head and neck cancer patients

**METHODS**

- Following IRB approval, a total of 21 patients with a diagnosis of oral or oropharyngeal squamous cell carcinoma were identified through the Brown Cancer Center.
- Each patient provided a serum and salivary sample for differential DSC processing along with a past medical history and social history questionnaire.
- Salivary samples were collected from 20 healthy control patients for comparisons.
- Previously analyzed serum thermogram data was used for healthy control comparisons.
- Salivary sample processing techniques were evaluated to determine reproducibility and standardization, after which a thermogram was determined for each of the salivary samples.
- Using previously outlined serum processing techniques, a thermogram was determined for each of the serum samples.
- Serum and salivary samples were compared to healthy controls, with thermogram changes evaluated as a function of tumor stage, location, and patient social risk.

**RESULTS**

A total of 21 head and neck cancer patients and 20 healthy controls provided samples for this study. As expected, nearly all of the sample patients possessed a history of tobacco abuse (81%), and many also had a history of alcohol abuse (48%). Among the smoking population, the average pack/year history was 57.5. The most frequent tumor location was tonsillar (38%), followed by oral tongue (29%), floor of the mouth (14%), and oropharyngeal mucosa (10%).

Serum thermograms were successfully created for each of these patients, exhibiting progressive changes from healthy controls, as shown in Figures 1 and 2. No apparent differences in thermogram signature were identified between subsets, with the exception of tonsillar disease. These lesions uniformly created thermogram signatures that were very similar to healthy controls, and differed as a group from all other subsites (Figure 3). Excluding these tonsillar specimens, the remainder of the subset thermograms demonstrated clear deviation from healthy controls, with displacement of the excess specific heat capacity peak towards higher temperatures. The extent of change correlated roughly with tumor T-stage. Most evident amongst tumors of the floor of mouth (Figure 4). No trends were identified when thermograms were evaluated based upon the nodal stage of each patient.

Salivary sample thermograms produced a much greater degree of deviation from one another, suggesting that sample processing and inherent variability are much more dynamic than their serum counterparts (Figure 5). Though individual thermograms could not be compared with pathology, when averaged by tumor stage broad trends were identified (Figure 6).

**DISCUSSION**

Differential scanning calorimetry provides a unique analysis of the plasma proteome. As opposed to mass spectroscopy and gel electrophoresis analyses, which focus on the identification of individual proteins in a sample, the thermogram provides a ‘signature’ that broadly displays protein composition. Analysis of these curves allows for a rapid determination of any broad deviations from the healthy control curve. Sample processing is also efficient and much less costly on a per patient basis than mass spectroscopy and gel electrophoresis, both of which are important characteristics of potential screening tests.

Several interesting conclusions were drawn from this pilot study. All head and neck cancer serum samples displayed visible deviations from healthy control thermograms with the clear exception of tonsillar lesions, suggesting a unique protein expression profile at this subsite. All other subsites demonstrated a general trend towards higher thermogram peak temperatures as a function of tumor T-stage. We propose that this shift is not due to a significant change in serum protein expression, but rather a change in the smallest 1% of the proteome. This protein fraction can bind to and alter the denaturation energy of its more abundant and higher molecular weight counterparts, namely albumin. This stabilizing interaction leads to the progressive deviation of thermogram peaks seen with larger lesions.

Salivary samples proved to be somewhat challenging. To our knowledge, this technology has not been previously applied to this sample medium, and a novel collection and processing protocol was created. Thermograms proved to be extremely volatile. While general trends could be seen after averaging all curves based upon tumor stage, individual curve analysis was not predictive of pathology. This difficulty was expected to a degree, as salivary protein composition is under the influence of several factors, including oral bacterial flora, dietary residue, and dental decay.

A larger patient population will be enrolled to further the application of calorimetry as a screening tool for head and neck carcinomas. Further refinements will be implemented in salivary sample processing in an attempt to control the outlined variables and volatility in thermogram production.

**CONCLUSIONS**

- Differential scanning calorimetry serum analysis produces reproducible trends in head and neck cancer patients.
- Thermogram signatures differ as a function of both tumor subsite and T-stage.
- Greater patient sampling is needed to further analyze the significance and validity of these trends as the first step to advancing cancer screening in this population.
- Salivary sample processing remains volatile, and sample processing protocols continue to be investigated.