G39 An ELISA Examination of Pro- Inflammatory Proteins in Human Blood Samples: A Potential Means for Investigating Diabetes in Postmortem Human Remains

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After attending this presentation, attendees will become familiar with cytokines and their relationship to the metabolic disorder diabetes mellitus.

This presentation will impact the forensic science community by demonstrating how blood cytokines may be successfully analyzed in human remains, and how results may be used to suggest diabetic condition. This is the first time that cytokine data has been applied to postmortem material to provide additional information that may advance human identification in forensic contexts.

The number of individuals suffering from diabetes has reached epidemic proportions globally and within the United States. In 2007, diabetes was cited as the seventh leading cause of death in the United States and was a contributing factor in another 160,000 deaths.[1] Forensic anthropologists must be prepared to recognize diabetes in skeletal remains as they would any other well-documented pathology. The purpose of this research is to investigate diabetes in postmortem material employing blood serum protein analyses.

Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycemia and disturbances in carbohydrate, fat, and protein metabolism, resulting from defects in insulin production and/or function.[2] Insulin regulation is crucial because this hormone is primarily responsible for transporting blood glucose into cells, providing a vital energy source. The present study will selectively focus on Type 2 diabetes, as it is most prevalent and the only form of diabetes documented in the William M. Bass Skeletal Collection.

The predominant theory explaining pathophysiology of diabetes involves activation of the immune system in response to metabolic stress, caused by over-nutrition. Normal physiological response to exogenous stress triggers inflammation of the affected tissue. When stress is acute, inflammation has positive effect, resulting in cell repair and tissue regeneration; however, when the condition is chronic, like obesity and insulin resistance, inflammation may become deleterious and maladaptive.[3] Researchers have identified that patients suffering from Type 2 diabetes demonstrate inflammation in tissues related to adiposity and glucose handling.[4] Studies have uncovered autoimmune inflammation in the insulin-producing pancreatic β-cells of diabetics. Cytokines are cell-signaling proteins that serve as primary mediators of immune responses. Many cytokines are secreted by adipocytes, demonstrating a link between obesity and insulin resistance. Pancreatic inflammation has been termed “insulitis” and may be is characterized by elevated levels of leukocytes and cytokines in the blood stream.[5] Biomarkers of inflammation, like cytokines, can be quantified and may be used as proxy variables to track the progression of insulin resistance.

The William M. Bass Skeletal Collection provides a unique opportunity to investigate diabetes in an extensively documented modern population. Blood sample collection was initiated in 2008 by the UT Forensic Anthropology Center for donated human remains. For this research, four preliminary samples were selected: two known diabetics and two non-diabetics who were pair-matched based on demographic characters (age, sex, ancestry, and body mass index). Diabetic status must be assumed from self-reported medical history.

Blood samples were extracted from the bloodcard paper matrix using a repo-buffer solution. A Bradford Quantification Assay was used to test and verify sufficient amounts of proteins present in each sample. Samples were then subjected to an ELISA multiplex. ELISA analyses determine the presence and concentration of an unknown biomarker. The multiplex investigated 26 pro-inflammatory cytokines. Elevated values were determined in eleven cytokines, six of which warranted statistical testing. Significant difference between the diabetic versus non-diabetic samples was assessed with univariate ANOVAs with a single fixed factor. Assumptions of normality and variance were tested and proven with a Shapiro-Wilks and Levene’s test (respectively). To avoid Type 1 error, a Bonferroni Correction was applied (modified α=0.008). After adjusting the alpha, one cytokine (IL-8) significantly differentiated between sample groups (α=0.001); however, another cytokine (MCP-1), demonstrated a tendency toward significance prior to Bonferroni adjustment (α=0.019) which may be recognized given a larger sample size. Both of these cytokines show high correlation with insulin resistance, and are reported to interact with bone cells

Results from this preliminary study indicate that cytokines may be successfully tested in postmortem samples. Data may be used to suggest diabetic status in unknown remains.

References:

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