Characterizing an Aberrant Band on Serum Protein Electrophoresis

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ABSTRACT

For decades one of the most widely adopted and extensively used methods to measure serum proteins in the clinical laboratory was serum protein electrophoresis (SPEP). This method involves the separation of large molecules based on size and charge across a solid matrix (agarose gel) maintained in a vertical or horizontal plane. The resolution and surface to volume ratios are low in SPEP. More recently, hospital laboratories are using capillary electrophoresis (CE) for the measurement of serum proteins mainly due to its high separation efficiency, short analysis time, low waste generation, and versatility. CE separates large and small molecules based on size and charge in a capillary tube. With CE, band resolution is high as well as the surface to volume ratios. While CE has many advantages as a technique, it can lead to the development of an aberrant band appearing between the beta and gamma regions. Currently, this band is being labeled as a non-specific band, even though the true identity of the band remains unknown. In this study, we identified 31 samples displaying an aberrant band between the beta and gamma regions on CE during a 6 month period. All samples were divided into two aliquots and frozen at -20°C until subsequent analysis. One aliquot was treated with thrombin and re-assayed using capillary electrophoresis and the other aliquot was used to perform western blot. Results indicated that twenty-six of the thirty-one samples showed the presence of CRP, fibrinogen like proteins (FGLs) or fibrinogen or combination of two. Fourteen samples showed the presence of CRP, twelve samples showed the presence of FGLs and five samples showed the presence of fibrinogen. The researchers concluded that the band identity is more related to acute phase proteins; CRP or FGL, or fibrinogen.

INTRODUCTION/OBJECTIVE

Electrophoresis is one of the most frequently used techniques in research laboratories and has become a commonplace technique in clinical laboratories to aid in the diagnosis of protein abnormalities. In its most basic definition electrophoresis refers to the movement of particles in a medium due to an external electrical field applied. The size and charge of the molecule determine the velocity of movement; therefore, different molecules that have the same charge but different sizes can be separated based on their charge and size. Recently, capillary electrophoresis has become increasingly popular in the clinical laboratory due to greater efficiency and shorter turn around times. However, this has been at a cost, specifically an aberrant band between the beta/gamma regions is appearing on some samples. The purpose of this study is to characterize the identity of the aberrant band appearing on capillary electrophoresis.

MATERIALS/METHODS

Thirty-one samples were identified to have an aberrant band on CE over a 6 month period. The identified samples were frozen at -20°C for subsequent analysis. All samples were thawed and separated into two aliquots. One aliquot was used for Western blotting and another for CE after thrombin treatment. Immunofixation electrophoresis results were also retrieved for each sample. This work has been approved by the UAB Institutional review Board: IRB-300000014.

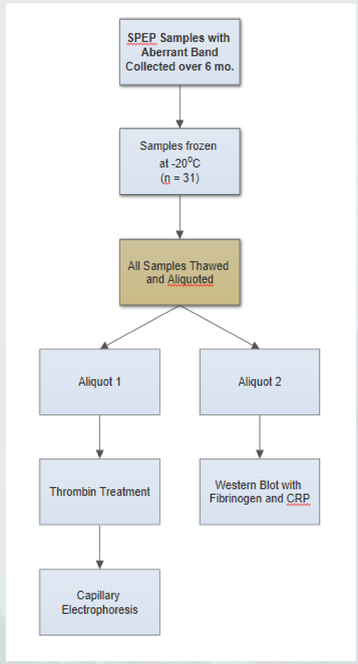

Figure 1: Research Design

RESULTS

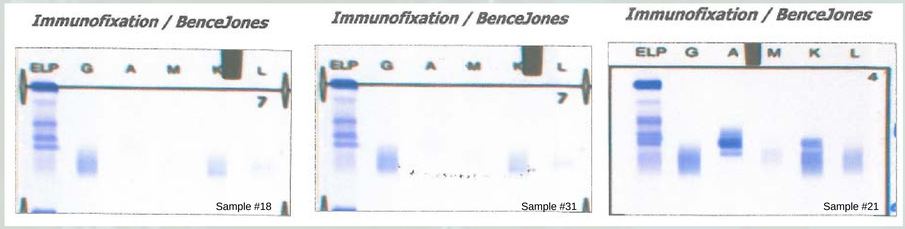

Figure 2: Immunofixation for samples 18, 31, and 21

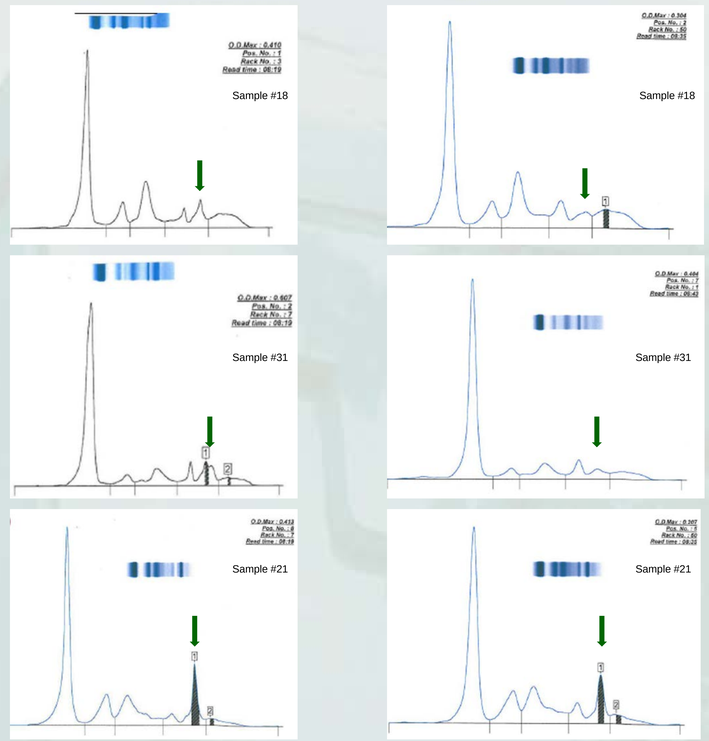

Figure 3: Examples of CE before and after thrombin treatment

CONCLUSION

Results in this study showed the presence of c-reactive protein (CRP) or fibrinogen like proteins as causes of the aberrant band in addition to fibrinogen due to an incompletely coagulated sample. The researchers concluded that the band identity is related to acute phase proteins (CRP or FGLs) or to fibrinogen.

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