Quantitative active and total kallikrein assay in human vitreous by LC/MS and its potential application in diabetic macular edema (DME) patients

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Diabetic macular edema (DME) is the most common cause of vision loss for people with diabetes, characterized by the accumulation of excess fluid in the extracellular space of the retina of the macular area. Current treatments, such as lasering, corticosteroids, intravitreal anti-vascular endothelial growth factor (VEGF) or surgery have varied results among patients, with only ~50% of VEGF recipients regaining vision after months of therapy. Recent studies have shown that the Plasma Kallikrein-Kinin (PKK) pathway and VEGF pathways are independent, with the PKK pathway correlating more closely with the protein levels. Based on these findings, an immunocapture LC-MS/MS method using an anti-kallikrein antibody was developed with a calibration range of 2–500 ng/mL to measure active kallikrein, and a direct digestion LC/MS method was developed with a calibration range of 25–500 ng/mL to measure total (active + prekallikrein) kallikrein, in human vitreous. The standard curves were linear throughout the calibration ranges. Intra and inter-assay precision and accuracy of the quality controls and patient samples were acceptable. By leveraging immunocapture, we can monitor active kallikrein separately from total kallikrein. Combining immunocapture with direct digestion, we can calculate the active and total kallikrein ratio, which provides information on the conversion and upregulation of active kallikrein in disease states. This novel LC-MS/MS method allows us to assess the ocular disease pathology previously unobserved in the DME pathway.

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