Quantitative Analysis of N-Glycans in Human Blood Serum Derived from Patients with Moderate to Severe Traumatic Brain Injury using LC-MS/MS

Kaitlyn Donohoo[1], Mona Goli[1], Byeong Gwan Cho[1], Alireza Banazadeh[1], Firas Kobaissy[2], Ryan Morgan[1], Jingfu Zhao[1], Endre Czeiter[3,4,5], Krisztina Amrein[3,4,5], Andras Buki[3,4,5], Sakshi Gautam[1], Wenjing Peng[1], Mondello Stefania[6,7]*, Yehia Mechref[1]*

[1]Chemistry and Biochemistry Department, Texas Tech University, Lubbock, Texas; [2]Department of Psychiatry and Neuroscience, McKnight Brain Institute, University of Florida, Gainesville, FL; [3]Department of Neurosurgery, University of Pecs, Pecs, Hungary; [4]János Szentágothai Research Centre; University of Pécs; [5]MTA-PTE Clinical Neuroscience MR Research Group; [6]Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Messina, Italy; [7]Oasi Research Institute-IRCCS, Troina, Italy.

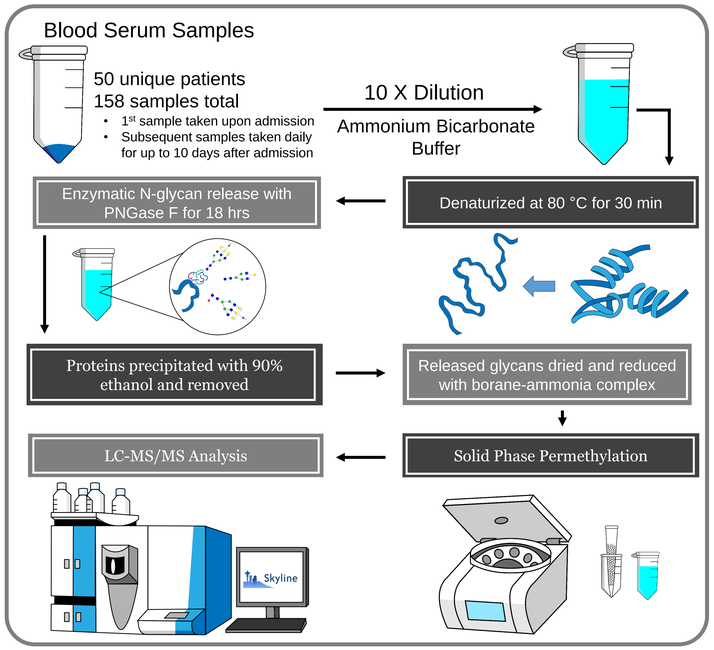
Introduction

Traumatic Brain Injury (TBI) is a major and increasing global health challenge. It is estimated that approximately one third of injury related deaths are caused by TBI. The molecular mechanisms underlying TBI pathophysiology are both complex and varied and may include aberrant glycosylation of proteins. We hypothesized that alterations of N-glycan expressions could represent important contributors to the overall pathophysiology of the disease, and be potential biomarker candidates to assess the injury severity as well as novel therapeutic targets. With these aims in mind, and to acquire a better understanding of their pathogenetic role, we investigated the expression of N-glycans in human blood serum from patients with moderate to severe TBI.

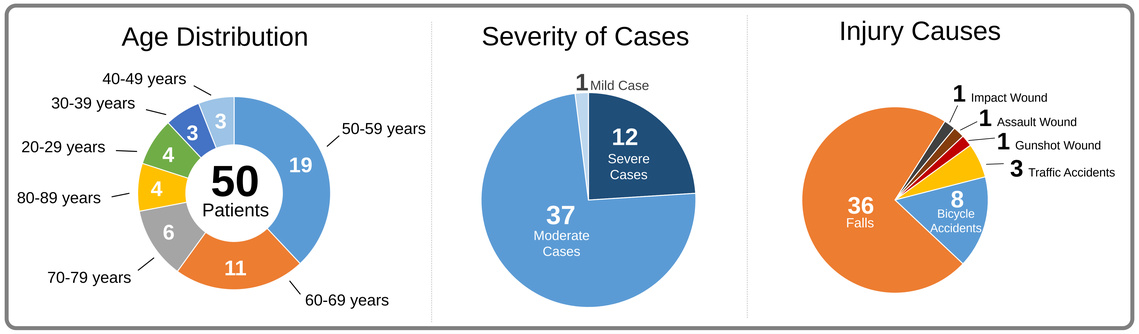
Method

Fifty patients with moderate to severe TBI (admission Glasgow Coma Score [GCS] 3-12) were included. Blood samples taken at the time of hospital admission and daily up to 10 days after injury and analyzed. Briefly, 10 µl of human blood serum was diluted with 90 µl of 50 mM ammonium bicarbonate buffer. After denaturation at 80 °C for 30 minutes, 1 µl of PNGase F (500 units/µl) enzyme was added to the sample and incubated at 37 °C for 18 hours. Proteins were then precipitated by adding 90% ethanol. The released glycans were dried and reduced by adding 10 µl of borane-ammonia complex, followed by solid-phase permethylation. Permethylated N-Glycans were analyzed using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS).

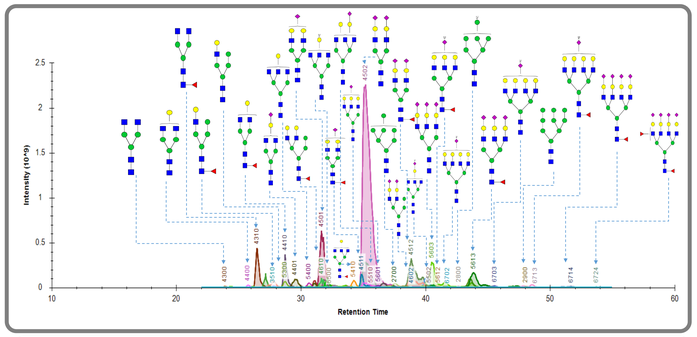
Workflow



Patients Clinical Information



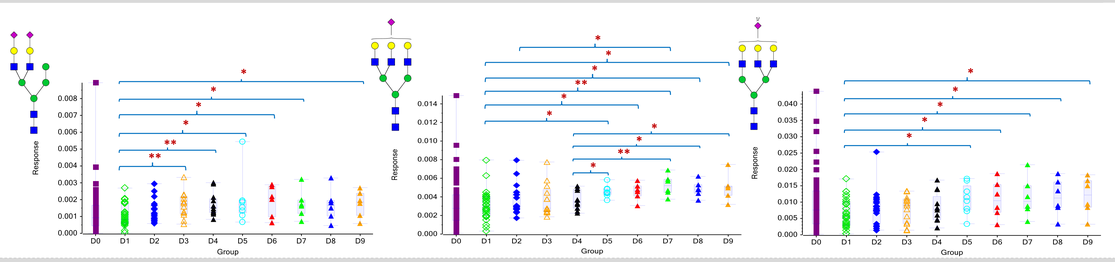
Extracted Ion Chromatogram of Permethylated N-glycans Derived from Pooled-HBS TBI Samples by using Skyline



Longitudinal Study of Glycan Expression Changes in TBI Patients

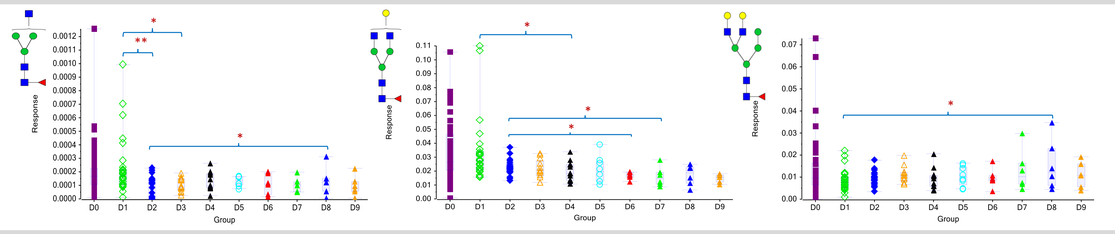
Sialylated Structures

In all figures: * is used for *p* value <0.05, ** is used for *p* value < 0.01, and *** used for *p* value < 0.001



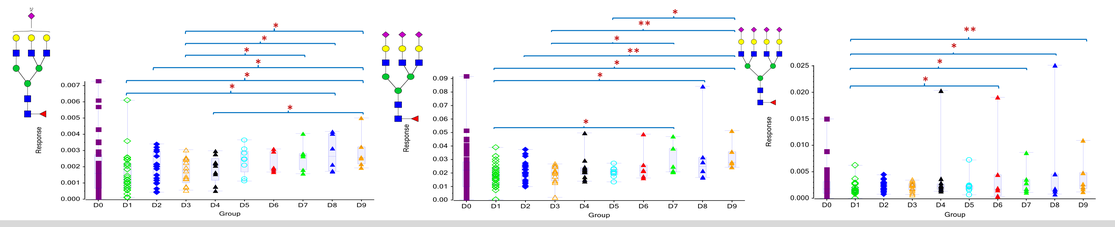
Fucosylated Structures

In all figures: * is used for *p* value <0.05, ** is used for *p* value < 0.01, and *** is used for *p* value < 0.001



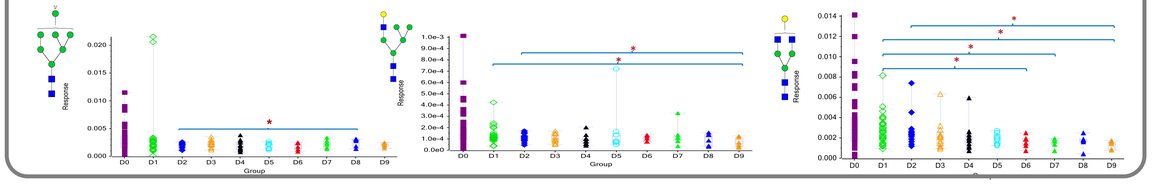
Sialylated & Fucosylated Structures

In all figures: * is used for *p* value <0.05, ** is used for *p* value < 0.01, and is *** is used for *p* value < 0.001

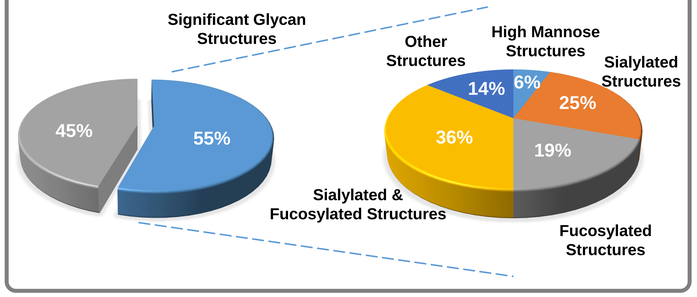


High Mannose & Other Structures

In all figures: * is used for *p* value <0.05, ** is used for *p* value < 0.01, and *** is used for *p* value < 0.001



Distribution of Different Glycan Types Among TBI Samples



Conclusion & Future Work

- ✓ According to the initial results, glycan expression in TBI patients showed significant changes over the days past the initial injury, suggesting that TBI leads to changes in glycan expression.
- ✓ A total of 55% out of all N-glycan structures were found to have significantly different expressions across multiple days. The majority of them demonstrated this differential expression between the early and late stages of TBI.
- ✓ Interestingly, more than half of the significant structures were found to be sialylated and/or fucosylated. Although the result is not conclusive yet, it is reasonable to postulate that these significant differences in glycan expressions might help to better comprehend the prognosis of TBI.
- ✓ Further investigation into the relationship between TBI pathophysiology and glycan expression should be conducted to determine the impact that glycan expression has on TBI and TBI patient recovery.

Acknowledgment

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