Label -Free Proteomics of Severe Acute Hepatitis of Unknown Origin in Children ByHigh-Resolution Mass Spectrometry

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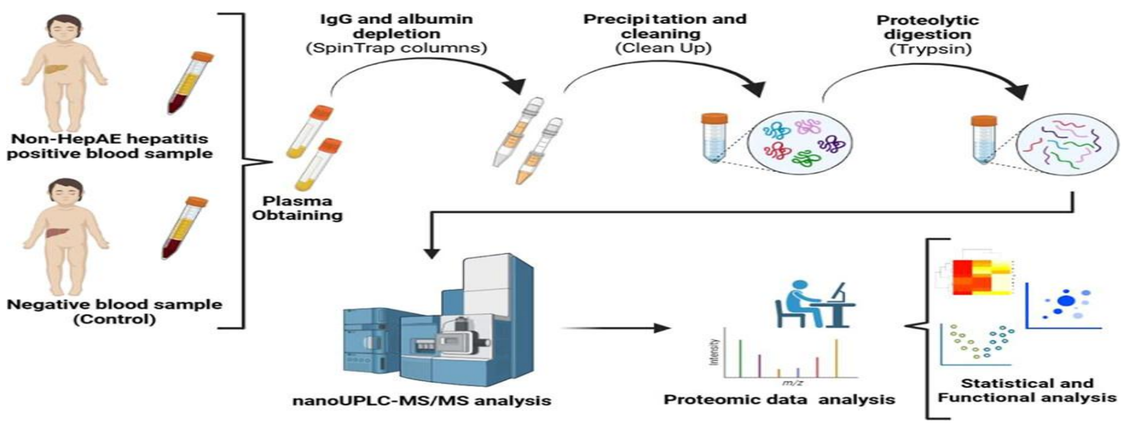
Introduction:

"Acute hepatitis is a sudden inflammation of the liver. In 2022, the WHO reported cases of severe hepatitis in children of unknown cause (non-HepA-E), with rapid progression to liver damage and the need for transplants. Routine tests for common hepatitis and autoimmune causes were negative. Proteomic studies are vital to uncovering the disease's causes, identifying biomarkers, and exploring treatments."



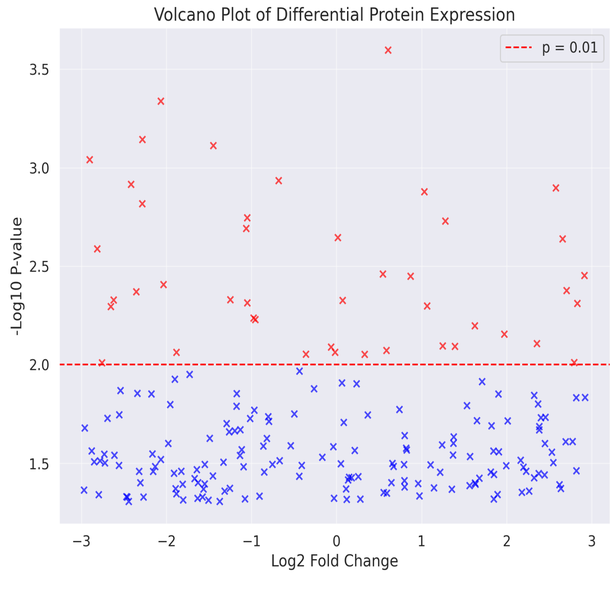
Aims of the Study:

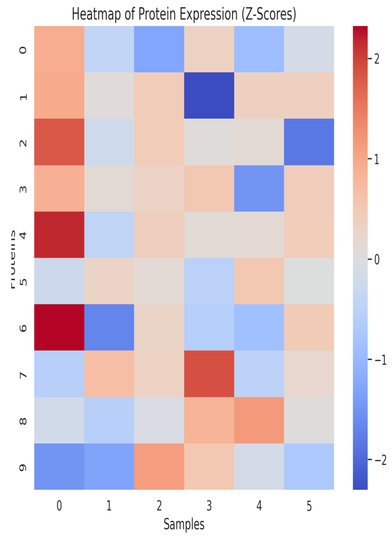
To analyze plasma samples from children with and without non-HepA-E hepatitis using high-resolution, label-free proteomics. To identify differentially expressed proteins (DEPs) and pathways involved in the disease process. To investigate the potential role of viral proteins as causative agents or triggers of immune dysregulation.

Material and Methods :

- **Experimental Design and Samples**: A clinical case study was conducted on non-HepA-E hepatitis. Plasma samples were collected from two female children: a 13-month-old positive for hepatitis and a 16-month-old negative control
- **Sample Preparation:** - *Depletion & Precipitation:* Albumin and IgG were removed using a SpinTrap Kit. Proteins were quantified using a BCA assay. - *Proteolytic Digestion:* Samples underwent reduction, alkylation, and digestion with trypsin. Processed samples were concentrated and stored at −80 °C.
- **Proteomic Analysis:** Peptides were separated using UPLC coupled to a SYNAPT XS MS/MS system. A multidimensional fractionation strategy was employed, and data were acquired in UDMSE mode.*
- **Proteomic Data Analysis:** Proteins were identified using the bioinformatics tools like Progenesis QI and UniProt database, with strict confidence thresholds (>99%). Perseus software was used for statistical filtering and analysis
- **Statistical and Functional Analysis:**

Provide a brief explanation of statistical tools used (e.g., heat map, volcano plot, Pearson correlation).Discuss protein-protein interaction analysis and its relevance to understanding disease mechanisms.



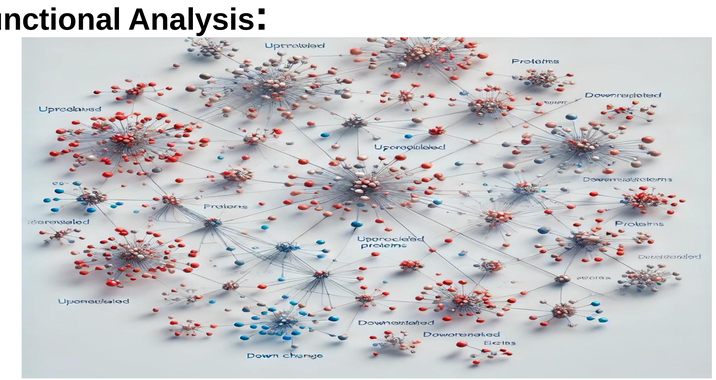


Results:

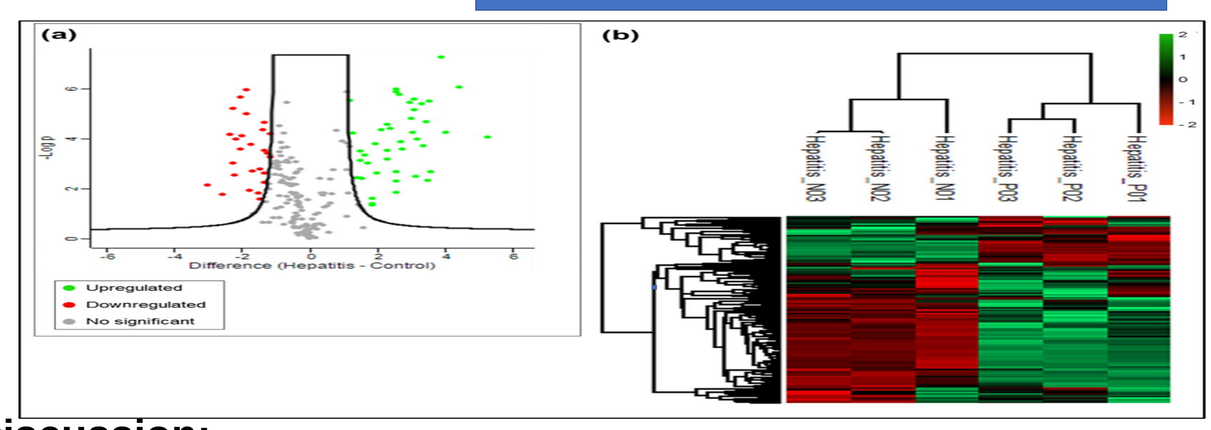
Proteomic Data & Statistical Analysis: Out of 206 proteins identified, 72 were differentially expressed (45 upregulated, 27 downregulated) in hepatitis non-HepA-E samples. Key upregulated proteins included immunoglobulins and viral replication factors, while downregulated proteins involved the complement cascade and apolipoproteins. Statistical analysis showed significant changes (q-value < 0.01).

Protein-Protein Interactions & Functional Analysis:

Downregulated proteins interacted with coagulation regulators, while upregulated apolipoproteins linked to coagulation. Immune-related proteins showed strong interactions, indicating immune system dysregulation, especially in liver-related pathways.



PPI The nodes represent proteins, with red nodes showing upregulated proteins and blue nodes showing downregulated proteins

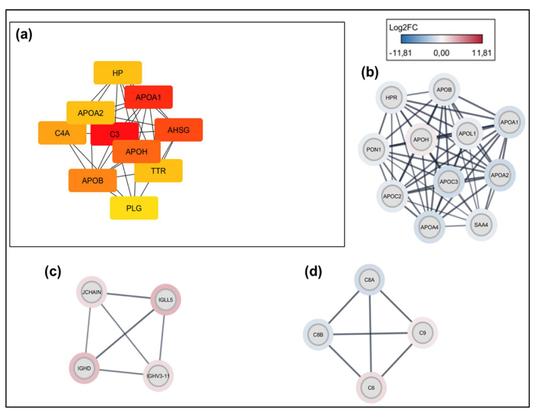


Discussion:

In our study, we identified key proteins upregulated in non-HepA-E hepatitis, including Gremlin-1 (GREM1), which may contribute to liver fibrosis by increasing TGF-β signaling. Other proteins, such as SERPINF2 and TRAF3IP1, are linked to immune responses and fibrosis progression. LGALS3BP was elevated, suggesting its role in fibrosis and liver disease severity. Additionally, downregulation of apolipoproteins and antioxidants like SOD3 indicates potential disruptions in lipid metabolism and increased oxidative stress, which could worsen liver damage. These findings highlight potential biomarkers and mechanisms involved in liver damage and fibrosis.

Conclusion:

Label-free proteomics revealed critical pathways and proteins involved in non-HepA-E hepatitis. Gremlin-1 and immune proteins may serve as biomarkers or therapeutic targets. Viral proteins suggest a post-infectious or immune-mediated disease process. This approach offers a roadmap for understanding rare liver diseases and developing targeted therapies.



Future Directions:

Expand study to include larger, diverse samples. Explore therapeutic targets and preventive strategies for liver fibrosis and severe hepatitis progression

References:

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3. Khader, S.; Foster, I.; Dagens, A.; Norton, A.; Sigfrid, L. Severe acute hepatitis of unknown a etiology in children-what is known? BMC Med. 2022, 20 (1), 280.
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