IPD Project Details

Project ID: IPD3343

Project Title: Semen proteome analysis comparing the persistent effects of different SARS-COV-2 variants on male reproductive system in recovered men

Description: After the initial COVID-19 wave, India experienced a second COVID-19 wave in March 2021, which was driven by the delta variant. By January 2021, India has also begun its vaccine campaign. Therefore, semen samples from recovered patients who were infected during the two waves of COVID-19 in India were obtained to study the impact of variants on the male reproductive system. We compared samples from the second wave with those of first wave in India. We also included control samples to the comparison.

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Sample Preparation: The semen samples were collected by masturbating into sterile containers after three days of no sexual activity. The samples were stored at 37 °C for 30 minutes after collection in order to liquefy them before analysis. For this, a specific room with laminar flow was designated, and all COVID-19 protocols were followed. Prior to processing the samples for proteomics and storing them at 80°C, the samples underwent a precautionary step of heat inactivation of the virus at 56°C for 30 min. The protein extracts were transferred to MASSFIITB (Mass Spectrometry Facility at IIT Bombay) for proteomics investigation. Using the Ghosh et al. 2022 technique, LFQ-based discovery proteomics was used to examine the long-lasting impact of viral infection on the male reproductive system. The proteome data were collected in this work using a high-resolution Orbitrap Fusion Tribrid Mass Spectrometer connected to a simple nano-liquid chromatography (LC) system. The LC-MS/MS runs parameters were as previously mentioned in Ghosh et al 2022.

Peptide Separation: 30 ?g of the protein was taken forward for digestion after reduction and alkylation using Tris carboxyethyl phosphine and iodoacetamide, respectively. Samples were then subjected to overnight digestion using Trypsin (0.25 ug/ul) in a ratio of 1:30. The digested peptides were then desalted using a C-18 STAGE tip. The final peptides were reconstituted in Milli-Q water with 0.1% formic acid and quantified before submission for MS run. Finally, 1 ?g of the peptide was injected for the LC–MS/MS run.

A high-resolution Orbitrap Fusion Tribrid Mass Spectrometer coupled to an easy nano-liquid chromatography (LC) system was used in this study to acquire the proteomic data in a data-dependent manner.

Protein Characterization: We compared the second wave samples with the first wave samples and control (published earlier; PRIDE ID: PXD026703). The datasets were processed using Label-Free Quantification based parameter in MaxQuant against Uniprot human database (downloaded on March 11, 2022). Furthermore, using the built-in search engine, Andromeda, only proteins having a maximum of 1% false discovery rate and unique peptides greater than 1 were selected to increase the reliability of the data obtained. Trypsin was set as the enzyme for digestion with maximum permissible missed cleavage as 2. Carbamidomethylation at cysteine was set as static modifications, and Oxidation at methionine was kept as variable modification.

Experiment Type: Shotgun proteomics

Species: Homo sapiens - 9606

Tissue: Semen (bto:0001230)

Cell Type:

Disease: Unknown

Instrument Details: Orbitrap Fusion (MS:1002416)

Protein Modifications: monohydroxylated residue, acetylated residue, iodoacetamide derivatized residue

PubMed ID: 38028760