Structural Prediction of Hepatitis E Virus X Domain to Limit Viral Infection: My Experience

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India experienced its first Hepatitis E viral outbreak in 1955-56 (Delhi) where 29000 cases and later during 1978-83 in Kashmir valley where ~52000 jaundice cases with 1700 deaths were reported. Recently in 2016, Shimla witnessed ~15000 cases of acute jaundice due to Hepatitis E virus (HEV) which took life of 21 residents. Such a huge magnitude of HEV infection was due to lack of specific antiviral drug and FDA approved vaccine which need to be addressed and solved as a researcher.

As a PhD student, working on Hepatitis E Virus, in the Department of Virology at the premier Medical Institute; Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh under the renowned clinical virologist Prof.(Dr.) R K Ratho, I actually witnessed the pain, stress and sufferings of patients and their expectations from the medical surgeons and scientists. My science journey daily started with a phone call to the Senior Residents of Liver ICU and Septic Labour Room (SLR):

Me: Hello,

Resident: Hello, Liver ICU/ SLR.

Me: Good morning Sir/Mam, this is Vikram from Virology department. Is there any HEV IgM/RNA positive patient admitted there?

Resident: Yes Sir, One patient X on Bed No. #. He is the liver transplant patient and now showing acute rejection (liver failure) due to HEV.

Me: Ok fine, Thank You, I will be there!

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* Mr. Vikram Thakur, Ph.D. Scholar from Postgraduate Institute of Medical Education and Research, Chandigarh, is pursuing his research on "Study of Inflammasomes in the Pathogenesis of Viral Hepatitis E Infection." His popular science story entitled "Structural Prediction of Hepatitis E Virus X Domain to Limit Viral Infection: My Experience" has been selected for AWSAR Award.

Unfortunately, this was my daily scenario for collecting blood samples from infected patients those are on central line for monitoring Heart Rate, Blood Pressure, Sodium and Dextrose levels. I always resist myself to collect blood from such patients who are in severe conditions and about to die. I was heavily stressed with the expectations of the patients for their cure, disease management and recovery.

Occasionally, I received calls from Liver ICU

Resident: Hi, This is Dr. # from Liver ICU.

Me: Good morning, Sir.

Resident: The patient from whom you collected blood yesterday, passed away last night. What about the other reports sir?

Me:……..

I experienced such painful and emotional incidents during my last 4 years as research student, that I always thought, why can't we simulate clinical conditions and do research for the betterment of the patients. Why always, I need to carry out research on every patients and treat them as experimental animal for our own interest. We are in the era of artificial intelligence and data science, with tonnes of scientific (genomic and proteomics) data in the form of sequences, clinical case reports, articles, etc. published and freely available to everyone. Why there is publish and perish attitude, where is the translational output? Swirling in these thoughts, I attempted to implement Structural Bioinformatics (Computational approach) to study HEV structure for better understanding of disease pathogenesis. This attempt helped me to get CSIR travel grant to attend 5 days training in European Bioinformatics Institute, Cambridge in United Kingdom (3^{rd}- 7^{th} Sept, 2018), where I equipped myself with all the possible algorithms, soft wares, servers, programmes, tools and implemented to predict "***Structure of Hepatitis E virus X domain to limit viral infection***" which might be useful to design the inhibitors. The research activity as follow:

The macro domain (X) is found to be ubiquitously present from Bacteria to humans and in many positive-strand RNA viruses like Rubella, Sindbis and SARS CoV. So far, HEV ORF1 X domain is known to interact with cellular ADP-ribose protein (involved in host pathogenesis). However, the detailed physiochemical characterization and putative refined structure of HEV X-domain with ligand binding active sites is not reported yet. So we proposed *in-silico* 3-D structure and functional characterization of HEV X-domain which will significantly improve our understanding of HEV pathogenesis and replication.

HEV X-domain sequence was retrieved from NCBI and characterized by ExPASY server. Crystallization probability was predicted by XtalPred, solvent accessibility by Raptor-X and disulphide linkages was predicted by DiANNA and DISULFIND server. Secondary structure was predicted by PredictProtein, SOPMA, PROFsec and Raptor-X Property server. 3-D structure was predicted by Phyre2, SwissModel, ITASSER and RaptorX and refined by ModRefiner server. Refined structure was validated by SAVES, RAMPAGE, QMEAN, Verify3D and ERRAT server. Finally the model was visualized in PyMol and the active binding site and ligands were predicted using RaptorX Binding tool and 3D ligandSite server.

The predicted HEV X domain model was found to be more stable (S^2> 0.8), ordered and compact. HEV X-domain represented high crystallization probability (score 1), two disulfide bond linkages (*Cys16-Cys145* and *Cys34-Cys91*), higher percentage of alpha helical (34%) and extended strand providing thermodynamically stable nature. RaptorX predicted 3D structure and identified HEV X-domain as putative phosphatase (resemblance with 1spvA). Refined structure with 98.1% residues in favoured region (Ramachandran plot), verified with Verify3D (85.44% residues 3D/1D score ≥ 0.2) server was acceptable predicted HEV X-domain. Multiplicity of 51 represented a deep binding pocket with 19 binding .residues for three different ligands viz. MES, APR and AR6.

Physiochemical properties suggested that the model is stable and in ordered form and has higher probability for crystallization which could be tried experimentally. Three ligands are predicted to bind with active binding site of HEV X-domain might prove to be a potential inhibitor to limit the HEV pathogenesis.

We communicated a Letter to Editor reporting Chronic HEV genotype-1 case in Journal of Hepatology (Deceased patient X). Although this was just the prediction and needs to be validated in the wet lab. An abstract of this work was submitted for International Conference INTERVIROCON to be held on 12th November 2018 – 14th November 2018 and full length article is in process for communicating in International Journal of Biological Macromolecules.

The main source of inspiration for this work is my Guide Prof. R K Ratho, Dr. Amin Sagar and EMBL-EBI mentors. I was fortunate to meet and talk with great scientist Prof. Dame Janet Thornton during UK training and her amazing words that "***Everything, even Brain is multidimensional and we should think 3-D dimensional to understand the protein structures***" continuously inspiring me to unlock the secrets behind the structures of viral proteins.

Keyword: Hepatitis E Virus, Structural Bioinformatics, Inhibitors, Patients, Research