# BIOPHYSICAL AND STRUCTURAL CHARACTERIZATION OF HEPATITIS A VIRUS (HAV) CAPSID PROTEIN VP1 GENERATED IN A HETEROLOGOUS EXPRESSION SYSTEM

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*by*

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*Submitted*

*in fulfilment of the requirements of the degree of*

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*to the*


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*Dedicated To my*

*Beloved Parents*
*&*
*Loving Husband*

For their unconditional love, endless support & encouragement

# CERTIFICATE

This is to certify that the thesis entitled **"Biophysical and Structural characterization of Hepatitis A Virus (HAV) capsid protein VP1 generated in a heterologous expression system"** being submitted by **Ms. Anshu Nain** to the **Kusuma School of Biological Sciences, Indian Institute of Technology Delhi** for the award of the degree of **"Doctor of Philosophy"** is a record of the bonafide research carried out by her, which has been prepared under my supervision and guidance in conformity with rules and regulation of the Indian Institute of Technology Delhi, India. The results prescribed in it have not been submitted in part or full to any other University or Institute for the award of any Degree/Diploma.

**Date:**

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***Anshu Nain***

# ABSTRACT

Hepatitis A Virus (HAV) is a quasi-enveloped picornavirus that causes acute hepatitis in humans and infects approximately 1.4 million individuals annually, not including the asymptomatically infected population. HAV, from being endemic in the Indian subcontinent and the far east, is slowly becoming a threat to public health. This is because improved socioeconomic conditions in specific pockets have drastically reduced the population of resistant individuals and increased the probability of outbreaks, with increased possibility of liver failure. The available vaccines against HAV are based on generation of infectious particles in culture and are therefore too expensive for universal vaccination efforts. HAV is very slow growing in culture and procuring large quantities of purified virus drives up the cost of generating inactivated or live attenuated vaccines. Under the given circumstances, generating cheaper subunit vaccines against HAV is a priority. Recombinantly generated HAV structural proteins may also be utilized for other usages, like development of effective diagnostic tests.

We attempted several strategies for recombinant production of one of the major capsid proteins, VP1, from HAV in *E. coli*. While several efforts resulted in the formation of soluble aggregates or co-elution of VP1 with the bacterial chaperone GroEL, correctly folded VP1 was eventually generated in a discrete oligomeric form upon purification of the protein from inclusion bodies and refolding. The oligomers resemble pentamers of VP1 from other picornaviruses and appear to have correct secondary and antigenic surface structure. These complexes can be utilized for understanding the molecular pathway of HAV capsid assembly and can also have potential biomedical usages in prevention and diagnostics of HAV infections.

# सारांश

हेपेटाइटिस ए वायरस (एचएवी) एक अर्ध-आच्छादित पिकोर्नावायरस है जो मनुष्यों में तीव्र हेपेटाइटिस का कारण बनता है और सालाना लगभग 1.4 मिलियन व्यक्तियों को संक्रमित करता है, जिसमें बिना लक्षण वाली संक्रमित आबादी शामिल नहीं है। एचएवी, भारतीय उपमहाद्वीप और सुदूर पूर्व में स्थानिक होने से धीरे-धीरे सार्वजनिक स्वास्थ्य के लिए खतरा बनता जा रहा है। ऐसा इसलिए है क्योंकि विशिष्ट इलाकों में बेहतर सामाजिक आर्थिक स्थितियों ने प्रतिरोधी व्यक्तियों की आबादी में भारी कमी ला दी है और लिवर फेलियर की संभावना के साथ प्रकोप की संभावना बढ़ गई है। एचएवी के खिलाफ उपलब्ध टीके संस्कृति में संक्रामक कणों की उत्पत्ति पर आधारित हैं और इसलिए सार्वभौमिक टीकाकरण प्रयासों के लिए बहुत महंगे हैं। एचएवी संस्कृति में बहुत धीमी गति से बढ़ रहा है और बड़ी मात्रा में शुद्ध वायरस की खरीद से निष्क्रिय या जीवित क्षीण टीके बनाने की लागत बढ़ जाती है। दी गई परिस्थितियों में, एचएवी के खिलाफ सस्ते सबयूनिट टीके तैयार करना प्राथमिकता है। पुनः संयोजक रूप से उत्पन्न एचएवी संरचनात्मक प्रोटीन का उपयोग प्रभावी नैदानिक परीक्षणों के विकास जैसे अन्य उपयोगों के लिए भी किया जा सकता है।

हमने ई. कोली में एचएवी से प्रमुख कैप्सिड प्रोटीनों में से एक, वीपी1 के पुनः संयोजक उत्पादन के लिए कई रणनीतियों का प्रयास किया। जबकि कई प्रयासों के परिणामस्वरूप घुलनशील समुच्चय का निर्माण हुआ या बैक्टीरियल चैपरोन ग्रोल के साथ वीपी1 का सह-संयोजन हुआ, सही ढंग से मुड़ा हुआ वीपी1 अंततः समावेशन निकायों से प्रोटीन के शुद्धिकरण और रीफोल्डिंग पर एक अलग ऑलिगोमेरिक रूप में उत्पन्न हुआ था। ऑलिगोमर्स अन्य पिकोर्नावायरस के वीपी1 के पेंटामर्स से मिलते जुलते हैं और उनमें सही माध्यमिक और एंटीजेनिक सतह संरचना होती है। इन परिसरों का उपयोग एचएवी कैप्सिड असेंबली के आणविक मार्ग को समझने के लिए किया जा सकता है और एचएवी संक्रमण की रोकथाम और निदान में संभावित जैव चिकित्सा उपयोग भी हो सकता है।

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# LIST OF ABBREVIATIONS

The following list gives account of various abbreviations and acronyms used throughout the thesis

| | |
|---|---|
| HAV | Hepatitis A Virus |
| VP | Viral protein |
| E.coli | Escherichia coli |
| UIP | Universal Immunization Programme |
| HPV | Human Papilloma Virus |
| CCMV | Cowpea Chlorotic Mottle Virus |
| FMDV | Foot and mouth disease virus |
| eHAV | enveloped Hepatitis A Virus |
| HAVcr-1 | Hepatitis A virus cellular receptor 1 |
| TIM-1 | T-cell immunoglobulin and mucin domain 1 |
| RNA | Ribonucleic acid |
| DNA | Deoxyribonucleic acid |
| cDNA | Complementary DNA |
| GST | Glutathione-S-Transferase |
| $H_6$ | Hexahistidine |
| LB | Luria-Bertani |
| IPTG | Isopropyl β-D-1-thiogalactopyranoside |
| PMSF | Phenyl methyl sulfonyl fluoride |
| SDS-PAGE | Sodium Dodecyl Sulfate-PolyAcrylamide Gel Electrophoresis |
| Ni- NTA | Nickel-Nitrilotriacetic acid |
| DTT | Dithiothreitol |
| EDTA | Ethylenediaminetetraacetic acid |
| HRP | Horseradish peroxidase |
| $ddH_2O$ | double distilled water |
| TEM | Transmission electron microscopy |
| FPLC | Fast protein liquid chromatography |

| | |
|---|---|
| LC/MS-MS | Liquid chromatography–mass spectrometry |
| Cryo-EM | Cryogenic electron microscopy |
| SEC | Size exclusion chromatography |
| ATP | Adenosine triphosphate |
| CHAPS | 3-cholamidopropyl) dimethylammonio)-1-propane sulfate |
| DLS | Dynamic light scattering |
| PBS | Phosphate-Buffered Saline |
| RT | Room Temperature |
| PVDF | Polyvinylidene fluoride |
| TBST | Tris-Buffered Saline with Tween |
| PBST | Phosphate-Buffered Saline with Tween |
| CD | Circular dichroism |
| ELISA | Enzyme-linked immunosorbent assay |
| SD | Standard Deviation |
| QELS | Quasi-elastic light scattering |
| TMB | Tetramethylbenzidine |
| CTF | Contrast transfer function |