Original article

Genomic Variation in Dengue Virus Non-Structural Protein 5 (NS5)

Saira Mushtaq, Malik Ihsan Ullah Khan, Muhammad Tahir Khan, Aneeqa Shahid, Rameesha Shafiq, Sarwat Jahan

ABSTRACT

Objective: To identify the most frequently repeated mutations in the NS5 protein in DENV RNA isolated from dengue patients in the province Punjab of Pakistan.

Methodology: The study was conducted at Aziz Fatimah Hospital and Allied Hospital Faisalabad during the 2022 dengue outbreak. The temporal sampling method was applied while selecting confirmed dengue patients from both hospitals, and written consent was taken. 120 blood samples were selected in total and after quantification only 23 samples were found fit for whole genome sequencing. Data was uploaded to Torrent Suite Server 4.10 for analysis. Epi Data Analysis, a WHO-developed software program, was used to calculate and summarize mutation frequencies. Excel analysis was used to check for flaws in the data.

Results: There were a total of 199 mutations across the entire nonstructural protein 5 (NS5) sequence. NS5 mutations were found in 9 genomic isolates, with Sample (S) 17 having the highest number.

Conclusion: The presence of large number of mutations in the structure of NS5 protein may be used in designing specific anti-viral against it, for better management of DENV infection in local settings in future.

KEYWORDS: Dengue virus, Whole Genome Sequencing, mutation, NS5, Pakistan

INTRODUCTION

Dengue is a viral ailment propagated by mosquitoes that is characterized by its acute and recurring nature. It is brought about by the dengue virus (DENV) and is commonly seen in tropical regions.[1] In the last five to six decades, the incidence of dengue has risen by a factor of thirty. Presently, around 390 million individuals globally acquire dengue fever year.[2] National Institute of Health (NIH) Islamabad, reported the number of dengue fever in Pakistan to be 22,938 in 2017, over 3,200 cases in 2018, 24,547 patients in 2019, and 3,442 cases in 2020.[3]

Dengue viruses (DENV), belonging to the Flaviviridae family and Flavivirus genus, induce a dengue infection that manifests as high fever, joint and muscle pain, vomiting, exhaustion, myalgia, skin rash, hemorrhagic episodes, abdominal discomfort, and circulatory shock.[4] DENV viruses are enveloped RNA viruses with a single-stranded genome. The open reading frame (ORF) of the DENV genome, which spans approximately 11 kilobases and is flanked by the 5' and 3' untranslated regions, is encoded. The open reading frame (ORF) encodes a solitary polyprotein, which is further

Saira Mushtaq,[1] *MBBS, PhD*
Associate Professor
Malik Ihsan Ullah Khan,[2] *PhD*
Assistant Professor
Muhammad Tahir Khan,[3] *PhD*
Assistant Professor
Aneeqa Shahid, MBBS,[4] *M.Phil*
Sr. Demonstrator
Rameesha Shafiq,[5] *MBBS*
Resident Trainee Internal Medicine
Sarwat Jahan,[6] *MBBS, M.Phil*
Associate Professor
[1,4,6]Aziz Fatimah Medical & Dental College, FSD, PAK
[2]Institute of Molecular Biology and Biotechnology, The University of Lahore, LHR, PAK
[3]Research Scientist at the Zhongjing Research and Industrialization Institute of Chinese Medicine, LHR, PAK
[5]Shaukat Khanum Memorial Cancer Hospital, LHR, PAK

Correspondence:
Saira Mushtaq
sairasaad@yahoo.com

segmented into 7 non-structural proteins (NS-1, NS-2, NS-2B, NS-3, NS-4, NS-4B, and NS-5) and 3 structural proteins (C: capsid, M: membrane, and E: envelope).[5]

NS5 is a non-structural protein that is simultaneously massive and highly preserved, consisting of 899 amino acids. This protein is soluble in water and has two unique activities. One enzyme function is as an RNA-dependent RNA polymerase (RdRp), responsible for replicating the viral RNA. The other is to acts as an RNA methyltransferase, which protects the viral genome by adding a cap to the RNA, allowing for the translation of polyprotein.[6] NS5 and NS3 form an RNA replicase complex in the endoplasmic reticulum during viral replication.[7] Following replication, it disengages from NS3 and moves into the nucleus. The NS5 protein in humans interacts with multiple proteins that are part of the JAK-STAT signaling pathway and interferes with the signaling triggered by antiviral interferon.[8]

Considering the involvement of NS5 in viral replication, conducting a mutational analysis of this protein could provide a valuable understanding of its functionality and its potential application in the development of medicines and vaccines targeting the Dengue Virus. This study is a deliberate attempt towards that objective.

METHODOLOGY

This cross-sectional study was the PhD research project of University of Lahore (UOL). Ethical approval was taken from UOL with reference no: CRiMM/22/Research/143 in year 2022. Data was collected from Aziz Fatimah Hospital and Allied Hospital Faisalabad during the dengue outbreak of 2022, after taking permissions from higher authorities of both hospitals.

All confirmed patients with dengue fever older than 13 years and of both sexes were selected from the dengue wards of Allied Hospital and Aziz Fatimah Hospital by temporal sampling method from the months of August till December, 2022. A written consent was taken from them. The study did not include participants with comorbidities like heaptitis, chronic liver illness, typhoid fever, or malaria. Additionally excluded were patients who had dengue shock syndrome (DSS). It was determined that the patients had dengue infection based on the results of a positive polymerase chain reaction (PCR) test for DENV, a positive NS1-antigen test, or positive IgM antibodies for DENV. On Performa, the results of clinical examinations, laboratory tests, and other diagnostic procedures were documented along with the clinical history and examination findings.

120 Blood samples were collected from the dengue patients within 7 days of the onset of symptoms and centrifuged and stored. The GeneJET viral DNA/RNA purification kit (Cat no. K0821) was used to get viral RNA directly from the serum of DENV-positive patients. The extracted RNA was quantified by performing PCR and gel electrophoresis. After Quantification only 23 samples were found fit to be processed further for sequencing. DENV WGS sequencing was carried out on selected samples on an Ion 510 chip. Data was uploaded to Torrent Suite Server 4.10 once the prepared chip was put onto the Ion XL 5 sequencer for sequencing. EpiData Analysis, a software programme developed by the WHO, was used to calculate and summarize the mutation frequencies. Excel analysis was used to check the data for flaws.

RESULTS

After quantification 23 of 120 blood samples were sequenced. 199 mutations have been found over the entire protein sequence in toto. NS5 mutations have been identified in 9 of the samples.

The most frequent mutations observed were F881Y (n=5), V784I and D785N (n=4), and I873L (n=3). Frequency of mutations is high at the C-terminal when compared with N-terminal (Table 1).

The NS5 RdRP (aa 320–368) region harbored fifteen different non-synonymous mutations V320L, R325G, R325K, L327P, D344E, D344G, T346A, T346I, F348L, E356G, D359G, D359H, T362A, K364R, and A365P in different genomes of

DENV.

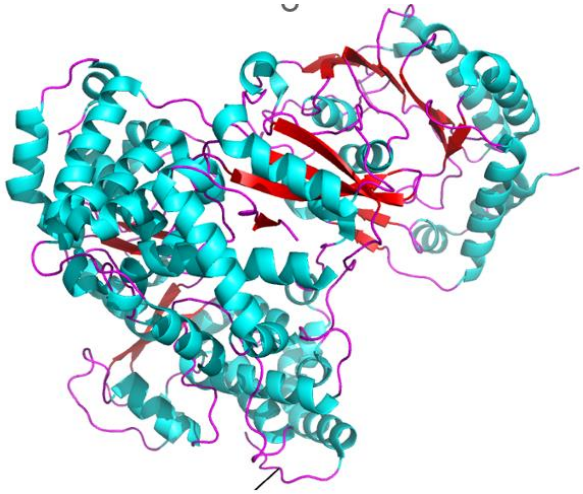
The NS5 has two cavities called A-cavity and Cavity B. In the current study, Cavity A position of NS5 Lys756, Glu807, Met809, and Leu seems highly conserved while mutation has been detected at two positions, E806G, M808T, and M808V in different genomes (Table 1). Furthermore, no alterations were seen in the conserved area of cavity B in the NS5 protein. N-terminal domain (aa 1–262) is called methyltransferase (MTase) domain harboring only 15 mutations in a very low frequency. The C-terminal domain of NS5 (aa273–900) contains the RNA-dependent RNA polymerase (RdRp) activity, has 164 mutations.

Table 1: Mutation frequency in NS5A proteins of DENV (n-23)

MUT	S.11	S.12	S.13	S.14	S.16	S.17	S.19	S.20	S.21	S.22	S.26	N(%)
F881Y	P			P		P			P	P		5 (21.7)
A774P				P		P	P			P		4(17.3)
A775P				P		P	P			P		4(17.3)
V784I				P		P	P			P		4(17.3)
D785N				P		P	P			P		4(17.3)
S210P	P			P		P						3(13)
V270I	P			P		P						3(13)
Y293N	P			P		P						3(13)
N297H	P			P		P						3(13)
K364R	P			P		P						3(13)
A365P	P			P		P						3(13)
R378K	P			P		P						3(13)
H435N	P			P		P						3(13)
I637A			P		P						P	3(13)
Q644K			P		P						P	3(13)
A648V			P		P						P	3(13)
S676N			P		P						P	3(13)
I687V			P		P						P	3(13)
A775V				P		P				P		3(13)
A775X						P	P			P		3(13)
N776I				P		P				P		3(13)
N776K				P		P				P		3(13)
I873L				P		P				P		3(13)
K130R				P		P						2(8.6)
N153S				P		P						28.6)
R430G					P						P	2(8.6)
N585S				P		P						2(8.6)
A616V				P		P						2(8.6)
T635N				P		P						2(8.6)
P636S				P		P						2(8.6)
V642A				P		P						2(8.6)
K647E				P		P						2(8.6)
T651V				P		P						2(8.6)
I669T				P		P						2(8.6)
V686M				P		P						2(8.6)
Q693L			P								P	2(8.6)
R728H				P		P						2(8.6)
E819G						P	P					2(8.6)
N820T				P		P						2(8.6)
L823K				P		P						2(8.6)
D825G							P			P		2(8.6)
L852F	P			P								2(8.6)
D296A				P								1(4.3)
T376A						P						1(4.3)
D689G				P								1(4.3)
P691Q				P								1(4.3)
A774V										P		1(4.3)
A775T				P								1(4.3)
K800T					P							1(4.3)
E806G						P						1(4.3)
M808T						P						1(4.3)
M808V							P					1(4.3)
V320L						P						1(4.3)
R325G						P						1(4.3)
R325K						P						1(4.3)
L327P				P								1(4.3)
D344G				P								1(4.3)
D344E						P						1(4.3)
T346A						P						1(4.3)
T346I		P										1(4.3)
F348L		P										1(4.3)
E356G		P										1(4.3)
D359G						P						1(4.3)
D359H						P						1(4.3)
T362A		P										1(4.3)
K364R		P										1(4.3)

N= frequency, %-percentages,

Figure 1. Structure of DENV NS5 protein and location of most common mutation



DISCUSSION

The NS5 is one of the three DENV water soluble protein (NS.1, NS.3 and NS.5). The NS5 protein is responsible for Viral gene replication and translation of polyprotein, thus acting as a perfect site for antiviral drug action and vaccine production against dengue.[9,10]

In our study N-terminal domain of NS5, called methyl transferase (MTase) domain harbored 15 mutations. The methyl transferase domain of NS5 protein binds to and degrades ERC1 protein of host and inhibits NF-kB activation. This activation is responsible for inflammatory cytokine secretion and cell migration, both needed for dengue virus pathogenesis.[11] The mutation found in our study could be explored further to study their effects on ERC1 binding function of NS5 protein. Another study showed that DENV disrupts host cell immune

response by binding its NS5 protein to the Polymerase associated Factor 1 complex (PAF1C). NS5 nuclear localization and C-terminal region of methyl transferase domain are crucial Interaction with PAF1C.[12] Mutations in this region might have an effect on this activity of NS5 protein.

In our study, the C-terminal domain of NS5 (aa273–900) contains the RNA-dependent RNA polymerase (RdRp) activity, has 164 mutations. A study conducted in Pakistan in 2022 showed I2762V, K2878E, H3047Y, K3061R, A3139V, S3167N, D3317E (amino acid) substitutions in sequences for NS5 in the C-terminal domain. The important polar–nonpolar amino acid substitution was observed to be R2921 in NS5.[13]

Our study lacks one specific amino acid mutations of N610G on the C-terminal domain of NS5, that are necessary for RdRp activity in flaviviruses. This mutation was identified as site under positive selection pressure in the full genome during the period of 2008 to 2013 in Pakistan, with a p-value of less than 0.08. The NS5-K861R mutation is present in DENV-2 strains from Pakistan between 2006 and 2022.[14]

Earlier study reported two mutations, Leu123Ile, and Leu879Ser in the NS5 protein.[15] Mutation Leu123Ile was also detected in the current study (Table 1). Another study conducted earlier revealed that the number of amino acid changes was higher in the NS5 gene, accounting for 3.7% of the total amino acid within the gene.[16] Same is observed in our study.

Despite efforts to prevent the spread of dengue, the prevalence of illnesses continues to rise globally. As a result, multiple dengue vaccines have been created and are now being tested in various stages of clinical trials, like Qdenga, a tetravalent dengue vaccine.[17,18] In this study we have identified mutations w through a comprehensive analysis of NS5 sequences from various DENV strains. The findings from this study provide valuable information on the genetic diversity of NS5 and its potential implications for the development of antiviral strategies.

Limitations: Dengue Virus infected patients came to hospitals late. Therefore, the viral load in their serum was low and because of this a lot of samples could not qualify for gene sequencing.

CONCLUSION

The presence of large number of mutations in the structure of NS5 protein may be used in designing specific anti-viral against it, for better management of DENV infection in local settings in future.

Conflict Of Interest: No known conflict of interest is present in this study.

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Author Contribution:

Saira Mushtaq: Study design, data collection, methodology, result compilation, article writing, revised and approved manuscript.

Malik Ihsan Ullah Khan: Supervision of research work, provision of resources during data collection, revised and approved manuscript

Muhammad Tahir Khan: Supervision of research, the main conceptualization of project, defining aims and objectives, revised and approved the manuscript

Aneeqa shahid: Data collection, manuscript writing, revise and approved the manuscript

Rameesha Shafiq: Data collection, data analysis, revised and approved the manuscript

Sarwat Jahan: Compiling of data, Manuscript writing, revised and approved the article

All authors are equally responsible for integrity of research work.

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