ORIGINAL ARTICLE ASIAN JOURNAL OF MEDICAL SCIENCES

Clinicoepidemiological study of dengue positive patients admitted to tertiary care hospitals in the Burdwan district, using molecular seroprofiling and enzyme linked immunosorbent assays of cytokines and chemokines

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Submission: 01-02-2023 Revision: 03-04-2023 Publication: 01-05-2023

ABSTRACT

Background: The World Health Organization views dengue fever as a significant worldwide public health threat in the tropics and subtropics. Dengue virus has four serotypes, DENG 1 to 4, which were responsible for outbreaks that occurred in other parts of the world from the 1980s until now. **Aims and Objectives:** Clinical manifestations and molecular and serological studies help determine the inflammatory response events associated with dengue severity. This study aimed to define the profiles of some cytokines and chemokines, as well as their molecular serotyping. **Materials and Methods:** Blood serum samples are collected, followed by serodiagnosis (NS1 and IgM) through ELISA, molecular serotyping detection of dengue by real-time PCR through SyBr green, and an immunological study of cytokines tumor necrosis factor-alpha and interferon-gamma (IFN-γ) on mother-hand chemokines CCL26 and MCP1 by ELISA. Statistical analysis was performed by SPSS (version 22). **Results:** The study examined 70 dengue seropositive samples, of which five were NS1 positive and 55 were IgM positive. After accounting for comorbidities and patients' demographic classifications, the 21–30 age group has a maximum positivity of 30%. The mean concentration level of IFN-γ is elevated at 1544.438 pg/mL. **Conclusion:** Dengue-positive patients have a high ischemic heart disease rate, among other diseases. An elevated circulating level of osteopetrosis may cause cancer, tuberculosis, and other serious diseases. In immunological aspects, IFN-γ elevation can be used to predict the severity of dengue infection.

Key words: Dengue; ELISA; Real-time-PCR; Serotype; DENG-3; Interferon-gamma; Cytokines and Chemokines; Co-morbidites

Access this article online

Website: http://nepjol.info/index.php/AJMS

DOI: 10.3126/ajms.v14i5.52001

E-ISSN: 2091-0576

P-ISSN: 2467-9100

INTRODUCTION

The dengue virus is an arthropod-borne virus that belongs to the family Flaviviridae and has four distinct serotypes (DEN-1, DEN-2, DEN-3, and DEN-4).[1] The World Health Organization (WHO) views dengue fever (DF) as a significant worldwide public health threat in the tropics and subtropics. Between 1960 and 2010, dengue cases rose by 30 times globally as a result of rapid population expansion, climate change, unplanned urbanization, ineffective mosquito

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control, frequent air travel, and a dearth of medical services.[2] There are 2.5 billion individuals in dengue-endemic areas, 400 million illnesses occur annually, and the fatality rate can exceed 20% in some places.[3] The first instance of dengue-like sickness to be documented in India happened in Madras in 1780, and the first epidemic of DF to be virologically proven in India took place in Calcutta and the Eastern Coast of India in 1963–1964.[4] All four dengue virus serotypes were responsible for significant outbreaks that occurred in other parts of the world throughout the 1980s and 1990s.[5]

Clinical manifestations of dengue virus infection are varied, ranging from asymptomatic sickness through DF to the severe condition known as dengue hemorrhagic fever or dengue shock syndrome (DHF or DSS).[6] DF (DHF) has been linked to oral mucosal damage DF since the 1950s, although oral characteristics are more typically linked to DF than to DF. The first pandemic broke out in Manila in 1954 and other nations began to experience this gradually. The global development of dengue infections may be explained by the fact that the more virulent genotypes of the virus are replacing the less virulent ones.[7-9] The E protein is organized on the surface of DENVs as 90 closely packed monomers that lay flat against the host cytoplasm. The E protein enhances viral entrance into host cells by binding to cellular receptors and facilitating the fusion of viral and cellular membranes. New DENV particles can either include processed M protein on release from infected cells, making them infectious or "mature."[8] The etiology of severe illness has been linked to non-structural proteins (NS1–NS5) produced in membrane-associated and secreted forms. NS1 is expressed on the surface of infected cells rather than being a component of the virion. When compared to patients with DF, patients with DHF had greater plasma levels of secreted NS1.[10] Furthermore, individuals who are at risk of developing DHF are identified by having higher free NS1 levels within 72 h after the commencement of the illness. Acute-phase samples from individuals with secondary dengue infections but not initial infections show very high levels of the NS1 protein. This shows that NS1, which is known to play a significant role in the pathogenesis of severe dengue infections, may help to create circulating immune complexes.[11] The Japanese encephalitis virus and the dengue virus both share antigenic epitopes. An important role in the spread of dengue is played by mosquitoes of the genus *Aedes aegypti*, the main and most significant vector; however, depending on the region, *Aedes albopictus* and *Aedes polynesiensis* may also function as vectors. For example, it has been discovered that *A. albopictus* can occasionally transmit dengue in Thailand, Samui Island, India, Singapore, and Mexico.[12]

To better understand the inflammatory response events associated with dengue severity, this study aimed to define the profiles of some cytokines and chemokines along with molecular serotyping. This might help identify biomarkers for the proper triage of patients with respect to infected serotypes.

Aims and objectives

To identify different serotypes of dengue virus and to evaluate the cytokines and chemokines concentration level in dengue positive patients.

MATERIALS AND METHODS

This institutionally based cross-sectional study was conducted at the Department of Microbiology, Burdwan Medical College, West Bengal, during the tenure of September to November 2022 (3 months). A total of 70 patient samples were collected from dengue IgM and NS1 seropositive patients. Samples were submitted with properly completed consent forms and clinical manifestations documented.

Ethical statement

This cross-sectional study has been approved by the Institutional Ethics Committee of Burdwan Medical College and Hospital.

Collection of samples

The blood samples were collected in a Clot Vial (red) after 30 min at the room temperature centrifuged the sample 1000 g in 30 s and separate the serum for further processing.

Serodiagnosis (NS1/IgM) through ELISA

All the patients' serum samples were collected and serodiagnosis of IgM and NS1 is done using STANDARD E Dengue IgM Capture ELISA manufactured by standard deviation (SD) Biosensor Healthcare Pvt. Ltd. for IgM testing and Oscar Medicare Pvt. Ltd. used for NS1 testing, as per manufactures instructions. Among all the IgM/NS1 dengue positive samples, 70 samples were selected based on their clinical manifestations.

Viral RNA isolation

The patient's RNA from the serum sample was isolated using the HiPurA Viral Automated RNA Purification Kit (HiMedia). RNA concentration was measured by measuring OD values using a spectrophotometer.

C-DNA synthesis

The extracted patient's RNA samples then convert into C-DNA by Prime Script™ 1st strand cDNA Synthesis Kit (HiMedia) as per the manufacturer's protocol.

Oligonucleotide design and synthesis

Dengue virus nucleotide sequences were obtained from GenBank. Primers were generated and tested for

quantitative real-time PCR (RT-PCR) after identifying potential target areas. Table 1 shows the final sequences of specific primer pairs.

Molecular detection of HINI by RT-PCR

The SyBr Green qualitative RT-PCR reactions that serotyped dengue (DENG1–DENG4) were performed using TB Green Premix Ex Taq II (Ti RNase H Plus), manufactured by Takara Bio. Each reaction contained 1 TB of Green Premix master mix and primers designed for a short sequence of polyprotein genes from different dengue serotypes, namely, DENG1, DENG2, DENG3, and DENG4. Bio-Rad CFX 96 RT-PCR conditions consisted of an initial denaturation incubation at 95°C for 2 min, followed by 38 cycles of alternating 95°C incubations for 5 s, 56°C incubations for 30 s, and 72°C incubations for 10 s. Fluorescence was detected after every 72°C extension incubation. This method was used to screen 70 clinical samples, allowing us to confirm the serotypes of dengue virus-infected individuals in the Burdwan district. The presence of the housekeeping gene Beta Actin was used as an internal test control to assess each specimen's DNA suitability for PCR amplification.

Immunological study of cytokines and chemokines by ELISA

The short-term plasmatic leakage caused by the host immune response is thought to be the crucial point in dengue physiopathology. Soluble immune mediators in the blood have been linked to severe illness outcomes such as shock and haemorrhages.[13,14] Among these are inflammatory cytokines such as tumor necrosis factor (TNF)-alpha and interferon-gamma (IFN-γ) and chemokines such as MCP-1,[15] which have all been found in dengue patients. We chose those two cytokines and two chemokines for this study to assess their concentration levels in dengue positive patients. Blood samples from 70 IgM/NS1 positive dengue patients were collected and stored at −80°C. Cytokines and chemokines were quantified through specific ELISA kit sets manufactured by Krishgen Biosystems, and the test was performed with the help of the manufacturer's instructions. This study measured TNF-alpha and IFN-γ under the cytokines group, and CCL26 (Eotaxin-3) and MCP-1 concentrations were measured under the chemokines group. All tests were performed, including standards, which were measured as per the manufacturer's guidelines, and OD values were measured by an ELISA reader (manufactured by J. Mitra). Figure 1 depicts the overall workflow of the project.

Statistical analysis

Analysis of all the data was performed by SPSS software (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Qualitative variables were expressed as mean±SD.

RESULTS

Patients clinical and demographic characterization

Among 70 dengue positive patients, the dominant age group is between 21 and 30 years and their positivity rate is 30 %. In Table 2, different age groups with their seroprevalence with positivity rate were described. In this study, we found that 1.43% of positivity rate in the above 61 years of age group which is lower than others. Among 70 patients, 50 were male patients and 20 female patients positive. In the sight of seroprevalence NS1 shows 5 positive, IgM shows 55 positive and combined showed 10 individuals positive.

In this study, among 70 dengue positive patients different comorbidities found. Diabetes mellitus (35.71%), hypertension (44.29%), ischemic heart disease (IHD) (54.29%), bronchial asthma (17.14%), chronic liver disease (15.71%), pulmonary tuberculosis (14.29%) found in those 70 dengue positive patients (Table 3).

In our total study time, we selected those 70 seropositive patients for molecular serotyping through RT-PCR of dengue virus circulating in Burdwan district, West Bengal. DENG3 (97.14%) is dominant with strong coinfection with DENG2 (82.86). DENG4 (5.71%) was found lowest prevalence (Table 4). DENG1 shows also mixed infection in some patients. In Supplementary file, the molecular serotyping with CT values is described.

Table 1: Degenerated primer sequences for the dengue group- and serotype-specific one-step SYBR Green-based RT-PCR assay

Serotype	Primer	Sequence (5' to 3')	Amplicon (bp)	Primer Conc.
DENG1	D1F	TCAATATGCTGAAACGCGCGAGAAACCG	482	10uM
	DS1	CGTCTCAGTGATCCGGGGG		
DENG2	D1F	TCAATATGCTGAAACGCGCGAGAAACCG	119	10uM
	DS2	CGCCACAAGGGCCATGAACAG		
DENG3	D1F	TCAATATGCTGAAACGCGCGAGAAACCG	290	10uM
	DS3	TAACATCATCATGAGACAGAGC		
DENG4	D1F	TCAATATGCTGAAACGCGCGAGAAACCG	389	10uM
	DS4	TGTTGTCTTAAACAAGAGAGGTC		

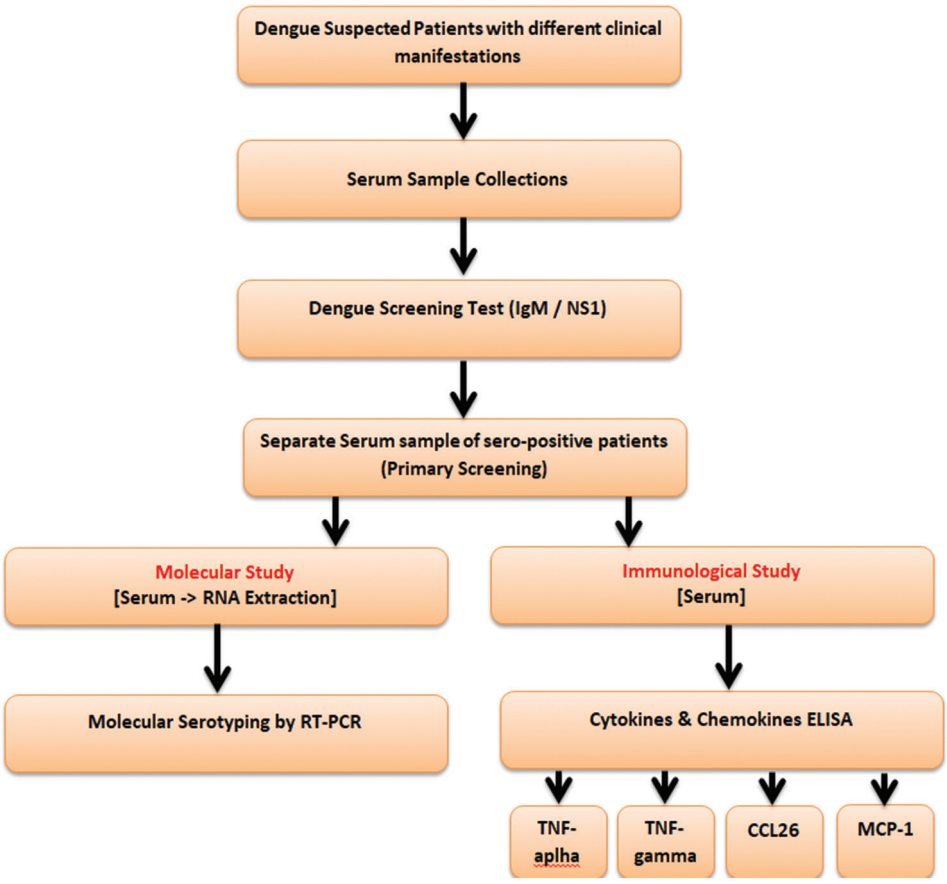

Figure 1: Workflow of molecular and immunological study among dengue seropostive patients

Table 2: Patients demographic characterization with IgM/NS1 prevalence

Age	NS1 positive	IgM positive	NS1+IgM positive	Male	Female	Total individual positive (%)
≤10 year	1	6	1	5	3	8 (11.42)
11–20 year	0	13	2	11	4	15 (21.43)
21–30 year	2	17	2	16	5	21 (30)
31–40 year	1	7	5	8	5	13 (18.57)
41–50 year	0	9	0	8	1	9 (12.86)
51–60 year	1	2	0	2	1	3 (4.29)
≥61 year	0	1	0	0	1	1 (1.43)
Total	5	55	10	50	20	70

Table 3: Comorbidities found in dengue IgM/NS1 positive patients

Comorbidities	Dengue positive (*n*=70)	Percentage
Diabetes mellitus	25	35.71
Hypertension	31	44.29
Ischemic heart disease	38	54.29
Bronchial asthma	12	17.14
Chronic liver disease	11	15.71
Pulmonary tuberculosis	10	14.29

Table 4: Molecular serotyping through RT-PCR of seropositive dengue patients

Total number of dengue positive samples (n=70)	DENG-1	DENG-2	DENG-3	DENG-4
Total positive	29	58	68	4
Percentage (n=70)	41.43	82.86	97.14	5.71

After the statistical analysis, the correlation between molecular serotyping DENG1-4 with IgM/NS1 serodiagnosis was described in Table 5 with their P-value. The statistical approach was made 2 sided asymmetrical manner. In this result DENG1 to DENG4, none of them shows a statistically significant because their value much higher than 0.05.

In this study, another statistical analysis was done and developed a correlation with comorbidities with four different dengue serotypes. In Table 6, there is a two-sided

crosstab statistical analysis was made and their P-value was given. No values were statistically significant in respect to comorbidities with dengue.

The mean concentration level of IFN-γ was elevated in the 70 patient's serum rather than TNF- α in the cytokines category. 15.44.438 pg/mL was the mean conc. of IFN-γ. In the chemokines category MCP-1, mean conc. was dominant than CCL26 among 70 dengue positive patients. The mean concentration level of MCP1 was 662.248 pg/mL. In total four IFN-γ, concentration was high than others. Cytokines and Chemokines mean concentration with SD were described and illustrated in Table 7 and Figure 2.

DISCUSSION

The four main serotypes of the dengue virus (DEN-1, DEN-2, DEN-3, and DEN-4) are arthropod-borne viruses that belong to the genus Flavivirus and family Flaviviridae.[1,16] Dengue is regarded by the WHO as a serious global public health threat in tropics and subtropics countries. Due to factors such a faster pace of population expansion, climate change, unplanned urbanization, ineffective mosquito control, frequent air travel, and a lack of medical services, dengue cases increased by 30 times globally between 1960 and 2010.[2,6,17] There are 2.5 billion people who live in dengue-endemic area,[2] and 400 million illnesses happen year with a fatality rate that exceeds 5–20% in some places.[3] More than 100 nations are affected by dengue illness, including Europe and the USA.[18] The first instance of dengue-like sickness to be documented in India occurred in Madras in 1780, and the first epidemic of DF to be virologically proven in India took place in Calcutta and the Eastern Coast of India in 1963–1964.[4] The clinical picture of dengue virus infection is varied and includes asymptomatic sickness, DF, and the severe illness known as (DHF/DSS).[6] Although oral symptoms are more typically linked with DHF than with DF, oral mucosal involvement is present in about 30% of patients.[7] Since dengue virus infection has a variety of clinical manifestations, a precise diagnosis is challenging and depends on laboratory confirmation. Since there is now no antiviral medication available, the problem typically resolves on its own.

In this study, DENG3 (97.14%) is predominant in Burdwan district but in among India in 51 research, dengue serotype information was available. The most common serotype in the four investigations from the northeast was DEN-3, which was followed by DEN-1 and DEN-2 serotypes.[19] All four dengue serotypes were found to be in circulation according to published studies from India, with DEN-2 and DEN-3 being the most often reported serotypes. More than one serotype was identified to circulate in two-thirds of the studies. An analysis showed that approximately one-fourth

Table 5: Correlation between serodiagnosis versus molecular detection of dengue with *P* values (Asym 2-sided)

SERO-positive (IgM/NS1)	DENG-1	DENG-2	DENG-3	DENG-4
P-value	0.139	0.139	0.755	0.016

Table 6: Correlation between comorbidities versus dengue serotypes with P values (Asym 2-sided)

Comorbidities	DENG-1	DENG-2	DENG-3	DENG-4
Diabetes mellitus	0.405	0.850	0.285	0.827
Hypertension	0.681	0.841	0.869	0.131
Ischemic heart disease	0.010	0.757	0.902	0.740
Bronchial asthma	0.985	0.428	0.514	0.011
Chronic liver disease	0.088	0.007	0.536	0.663
Pulmonary tuberculosis	0.552	0.517	0.558	0.523

*P<0.05 is typically considered to be statistically significant

Table 7: Cytokines and chemokines Conc. level in dengue positive patients

Total number of dengue positive samples (n=70)	TNF- α (pg/mL)	IFN-γ (pg/mL)	CCL26 (pg/mL)	MCP1 (pg/mL)
Mean conc.	354.925	1544.438	103.530	662.248
SD	140.061	568.098	44.156	255.882

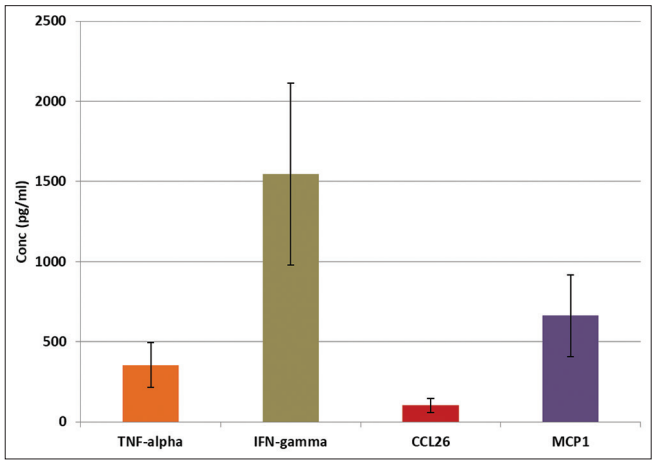

Figure 2: Concentration Level of different cytokines and chemokines among 70 dengue positive patients

of the cases were severe and that more than two-fifths of the laboratory-verified illnesses were secondary infections.[20] After statistical analysis, we found no significant relation in between molecular serotyping with serodiagnosis among 70 dengue positive patients. The P-value was much higher than 0.05.

An increase in TNF-α and MCP-1 can be used to predict the severity of dengue infections. This may assist in triaging patients as it may be difficult to assess the severity at the time of diagnosis. Early identification of more advanced disease together with more proactive management may result in favorable outcomes.[21] Studies have shown that increased mean concentration level of TNF-alpha among 70 dengue positive patients. IFN-γ is a main cytokine released by CD8 effector T cells, capable of blocking viral replication. IFN-γ is also a potent activator for phagocytic cells, increasing their activity as well as their ability to produce cytokines. IFN-γ has been shown to increase permeability of endothelial cells and to upregulate expression of TNF receptors on myeloid and epithelial cells, which can make them more sensitive to TNF-α exposure.[22,23] IFN-γ is vital to tumor surveillance by the immune system and a high correlation between IFN production and tumor regression has been seen in immunotherapy. It is anti-angiogenic, inhibits proliferation, sensitizes tumors cells to apoptosis, upregulates MHC class I and II expression, and stimulates antitumor immune activity. There have been mixed results as to the effectiveness of IFN-γ in the clinical treatment of various cancers.[24] IFN-γ activates alveolar macrophages, which are important in host immunity against M. tuberculosis. Coadministration of IFN-β and anti-TB to TB patients led to a reduction in cavitary lesion sizes and a decrease in the mycobacterial burden.[24] IFN-γ has been used in the treatment of scleroderma because of its antifibrotic activity, its ability to reduce collagen production *in vitro*, and to inhibit fibroblast cell proliferation. In most clinical trials, either subcutaneous or intramuscular administration of the drug has resulted in modest improvements in patients with the disease.[24] In our study, plasma levels of IFN-γ were not related to the presence of ascites, which are considered a marker of plasma leakage. The study's findings on the putative pathogenetic involvement of IFN- in the induction of plasma leakage were inconclusive. Due to this, it is impossible to draw definitive conclusions about how both cytokines contribute to the pathogenesis of dengue virus infection from the data.

Limitations of the study

Sequencing should be done to identify any mutation. We have not done it.

CONCLUSION

In this study, the clinical picture of dengue cases in Burdwan, along with the cytokines and chemokines responses. In the age group of 21–30 years were dominant and DENG3 prevalence higher than others. Coinfection of DENG2 and DENG3 occurs prominently after molecular serotyping through RT-PCR. IHD is a top most comorbidity found among dengue positive patients in Burdwan district. Higher level of IFN-γ may leads to cancer, tuberculosis, esteopetrosis, scleroderma, etc. which may a leads to serious threat for mankind. IFN-γ may play a prognostic biomarker for progression of such kind of disease after exposure of dengue virus infection. Our results suggested a downregulation of IFN-γ has traditionally been thought of as a pro-inflammatory cytokine that regulates anti-inflammatory responses. Therefore, lowering the pro-inflammatory cytokines could be a preventive measure and is hypothesized to be a possible drug target area.

ACKNOWLEDGMENT

Grateful to the Principal, Dean, and Medical Superintendent of Burdwan Medical College for allowing us to conduct the study; to all the staff of the Microbiology laboratory for cooperating with us to carry out the study; to all the participants of this study for their full-hearted support and cooperations.

REFERENCES

1. Kurane I. Dengue hemorrhagic fever with special emphasis on immunopathogenesis. Comp Immunol Microbiol Infect Dis. 2007;30(5-6):329-340.
   https://doi.org/10.1016/j.cimid.2007.05.010
2. Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, et al. Dengue: A continuing global threat. Nat Rev Microbiol. 2010;8(12 Suppl):S7-S16.
   https://doi.org/10.1038/nrmicro2460
3. Linares EM, Pannuti CS, Kubota LT and Thalhammer S. Immunospot assay based on fluorescent nanoparticles for dengue fever detection. Biosens Bioelectron. 2013;41:180-185.
   https://doi.org/10.1016/j.bios.2012.08.005
4. Gupta N, Srivastava S, Jain A and Chaturvedi UC. Dengue in India. Indian J Med Res. 2012;136(3):373-390.
5. Nimmannitya S. Dengue haemorrhagic fever: Current issues and future research. Asian Ocean J Paediatr Child Health. 2002;1:1-21.
6. World Health Organization. Dengue-Guidelines for Diagnosis, Treatment, Prevention and Control. New ed. Geneva, Switzerland: World Health Organization; 2009.
7. Thomas EA, John M and Bhatia A. Cutaneous manifestations of dengue viral infection in Punjab. Int J Dermatol. 2007;46(7):715-719.
   https://doi.org/10.1111/j.1365-4632.2007.03298.x
8. Guzman MG and Kouri G. Dengue: An update. Lancet Infect Dis. 2002;2(1):33-42.
   https://doi.org/10.1016/s1473-3099(01)00171-2
9. Rico-Hesse R. Microevolution and virulence of dengue viruses. Adv Virus Res. 2003;59:315-341.
   https://doi.org/10.1016/s0065-3527(03)59009-1
10. Libraty DH, Young PR, Pickering D, Endy TP, Kalayanarooj S, Green S, et al. High circulating levels of the dengue virus nonstructural protein NS1 early in dengue illness

correlate with the development of dengue hemorrhagic fever. J Infect Dis. 2002;186(8):1165-1168.
https://doi.org/10.1086/343813

11. Young PR, Hilditch PA, Bletchly C and Halloran W. An antigen capture enzyme-linked immunosorbent assay reveals high levels of the dengue virus protein NS1 in the sera of infected patients. J Clin Microbiol. 2000;38(3):1053-1057.
https://doi.org/10.1128/JCM.38.3.1053-1057.2000
12. Malavige GN, Fernando S, Fernando DJ and Seneviratne SL. Dengue viral infections. Postgrad Med J. 2004;80(948):588-601.
https://doi.org/10.1136/pgmj.2004.019638
13. Butthep P, Chunhakan S, Tangnararatchakit K, Yoksan S, Pattanapanyasat K and Chuansumrit A. Elevated soluble thrombomodulin in the febrile stage related to patients at risk for dengue shock syndrome. Pediatr Infect Dis J. 2006;25(10):894-897.
https://doi.org/10.1097/01.inf.0000237918.85330.b9
14. Srikiatkhachorn A, Ajariyakhajorn C, Endy TP, Kalayanarooj S, Libraty DH, Green S, et al. Virus-induced decline in soluble vascular endothelial growth receptor 2 is associated with plasma leakage in dengue hemorrhagic fever. J Virol. 2007a;81(4):1592-1600.
https://doi.org/10.1128/JVI.01642-06
15. Mao ZQ, Minakawa N and Moi ML. Novel antiviral efficacy of *Hedyotis diffusa* and *Artemisia capillaris* extracts against dengue virus, Japanese Encephalitis virus, and *Zika virus* infection and immune regulatory cytokine signatures. Plants (Basel). 2022;11(19):2589.
https://doi.org/10.3390/plants11192589
16. Halstead SB. Pathogenesis of dengue: Challenges to molecular biology. Science. 1988;239(4839):476-481.
https://doi.org/10.1126/science.3277268
17. Gubler DJ. Dengue and dengue hemorrhagic fever. Clin Microbiol Rev. 1998;11(3):480-496.
https://doi.org/10.1128/CMR.11.3.480
18. San Martin JL, Brathwaite O, Zanbrano B, Solorzano JO, Bouckenooghe A, Dayan GH, et al. The epidemiology of dengue in the Americas over the last three decades: A worrisome reality. Am J Trop Med Hyg. 2010;82(1):128-135.
https://doi.org/10.4269/ajtmh.2010.09-0346
19. Ganeshkumar P, Murhekar MV, Poornima V, Saravanakumar V, Sukumaran K, Anandaselvasankar A, et al. Dengue infection in India: A systematic review and meta-analysis. PLoS Negl Trop Dis. 2018;12(7):e0006618.
https://doi.org/10.1371/journal.pntd.0006618
20. Halstead SB. Dengue. Lancet. 2007;370(9599):1644-1652.
https://doi.org/10.1016/S0140-6736(07)61687-0
21. Jeewandara C, Gomes L, Wickramasinghe N, Gutowska-Owsiak D, Waithe D, Paranavitane SA, et al. Platelet activating factor contributes to vascular leak in acute dengue infection. PLoS Negl Trop Dis. 2015;9(2):e0003459.
https://doi.org/10.1371/journal.pntd.0003459
22. Stolpen AH, Guinan EC, Fiers W and Pober JS. Recombinant tumor necrosis factor and immune interferon act singly and in combination to reorganize human vascular endothelial cell monolayers. Am J Pathol. 1986;123(1):16-24.
23. Pandita R, Pocsik E and Aggarwal BB. Interferon-gamma induces cell surface expression for both types of tumor necrosis factor receptors. FEBS Lett. 1992;312(1):87-90.
https://doi.org/10.1016/0014-5793(92)81416-j
24. Miller CH, Maher SG and Young HA. Clinical use of interferon-gamma. Ann N Y Acad Sci. 2009;1182:69-79.
https://doi.org/10.1111/j.1749-6632.2009.05069.x

Authors' Contributions:
NC- Concept and design of the study, prepare the 1st draft of manuscript, preparation of manuscript; **TB-** Concept and Design of the study, Prepare the 1st draft of manuscript, Preparation of manuscript; **SN-** Prepare review of literature and preparation of manuscript; **AN-** Perform the laboratory test, prepare the result and interpretation; **NM-** Perform the laboratory test, prepare the result and interpretation; **SM-** Sample collection and documentation; **KD-** Sample collection and documentation.

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Source of Funding: ICMR-DHR Viral Research and Diagnostic Laboratory (VRDL), Department of Microbiology, Burdwan Medical College.
Conflicts of Interest: No! Conflict of interest is found elsewhere considering this work.