Pharmacogn J. 2024; 16(4): 923-926
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Original Article

Positive NS1 Antigen in Non-Dengue Virus Infection Serum: Possible Reasons for the Discrepancy with DENV PCR Results

I Gusti Agung Ayu Eka Putri Sunari[1], Aryati Aryati[1]*, Faradila Khoirun Nisa Hakim[2], May Fanny Tanzilia[3], Ida Parwati[4], Rismawati Yaswir[5], Budi Mulyono[6]

ABSTRACT

Background and Objective: A specific examination is required to distinguish between DVI and viral, bacterial, and parasitic illnesses because their clinical manifestations are nearly identical. Leukopenia and lymphocytosis are examples of non-specific tests that might be used to get a diagnosis. Non-structural protein 1 (NS1) antigen, anti-DENV antibody, or DENV-specific nucleic acid detection are more specific assays. **Methods:** Virus isolation or molecular analysis of the detection of DENV nucleic acid ribonucleic acid (RNA) using RT-PCR was used to make the conclusive diagnosis of DVI. The sensitivity of the DENV RT-PCR method ranges from 28.8 to 99%. NS1 antigen is used as an initial diagnostic option in primary health care because it has a high specificity value (100%). Researchers want to analyze the positivity in non-DVI samples that have been confirmed by real-time RT-PCR examination with semi-quantitative NS1 antigen examination. Patient population aged 1-65 years with acute fever <5 days. A total of 130 samples of non-DVI confirmed patients by RT-PCR were examined for NS1Antigen ELISA. **Results:** With a proportion of 3.08% of the total sample, the results showed that 4 NS1Antigen ELISA samples were positive. A negative DENV RT-PCR result could indicate either a true negative or a false negative. **Conclusion:** The type of PCR technology, the primer used, the existence or absence of a DENV mutation, the DENV serotype, and the presence of mismatched nucleotides can all affect variations in DENV PCR sensitivity.

Keywords: RT-PCR DENV, NS1Ag, Dengue Virus Infection.

I Gusti Agung Ayu Eka Putri Sunari[1], Aryati Aryati[1]*, Faradila Khoirun Nisa Hakim[2], May Fanny Tanzilia[3], Ida Parwati[4], Rismawati Yaswir[5], Budi Mulyono[6]

[1]Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo Academic Hospital, Surabaya, INDONESIA.

[2]Faculty of Medicine, Universitas Airlangga, Surabaya, INDONESIA.

[3]Department of Clinical Pathology, Faculty of Medicine, Universitas Ciputra, Surabaya, INDONESIA.

[4]Department of Clinical Pathology, Faculty of Medicine, Universitas Padjadjaran, Hasan Sadikin General Hospital, Bandung, IINDONESIA

[5]Departement of Clinical Pathology, Faculty of Medicine, Universitas Andalas, Padang, INDONESIA.

[6]Department of Clinical Pathology and Laboratory Medicine, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada - Dr. Sardjito General Hospital, Yogyakarta, INDONESIA

Correspondence

Aryati

Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo Academic Hospital, Surabaya, INDONESIA.

E-mail: aryati@fk.unair.ac.id

History

- Submission Date: 30-06-2024;
- Review completed: 28-07-2024;
- Accepted Date: 07-08-2024.

DOI : 10.5530/pj.2024.16.149

Article Available online

http://www.phcogj.com/v16/i4

INTRODUCTION

Fever is a common symptom of infectious diseases and is a primary reason patients seek medical attention. Vector-borne illnesses account for more than 17% of all infectious diseases.[1] In Indonesia, vector-transmitted diseases (VBD) have the potential to cause unusual health events.[2] Differential diagnoses of fever caused by vector-borne diseases include dengue virus infection (DVI), malaria, filariasis, chikungunya, Japanese encephalitis, and plague, among others.[1,2] Dengue hemorrhagic fever (DHF) was first reported in Indonesia in 1968, in the cities of Surabaya and Jakarta, with 24 fatalities at that time. The number of cases has since increased, parallel to the expanding dengue-endemic areas in Indonesia.[3,4)] This makes DHF, caused by the Dengue virus (DENV), a significant health challenge in the country and an endemic disease.[2,5]

The clinical manifestations of DVI closely resemble those of other viral infections, necessitating specific tests to differentiate it from other infections.[6] Identifying the infecting virus type is crucial for appropriate therapy and precise early detection, aiding in effective supportive management.[7,8] The diagnosis of DVI relies on the patient's clinical manifestations and various laboratory examination modalities, each with its own advantages and disadvantages. Therefore, these examinations are complementary to ensure an accurate and timely diagnosis.[9]

Several methods, including the detection of non-structural protein 1 (NS1) antigen, anti-DENV antibodies, and DENV-specific nucleic acids, can confirm a dengue virus infection. Given the limitations of each modality, combining several laboratory examination techniques is strongly recommended for diagnosing DVI(9). A definitive diagnosis can be achieved through dengue virus isolation or molecular analysis by identifying DENV ribonucleic acid (RNA) utilizing the Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) method.[10]

RT-PCR testing is advantageous due to its high specificity (99% to 100%) and variable sensitivity (28.8%-99%), depending on the PCR method.[10,11] However, RT-PCR has several drawbacks, such as the need for expertise, relatively high costs, and the requirement for a comprehensive set of sophisticated laboratory facilities. Consequently, PCR examinations are challenging to implement in primary care laboratories, particularly in Indonesia, and are not commonly performed.[12] RT-PCR examinations are necessary as confirmatory tests, especially in cases of DVI with positive NS1 antigen findings, considering the possibility of cross-reaction and hyperendemic areas.[9]

The most commonly used tests to support the diagnosis of DVI are antigen and antibody tests, which differ slightly from DENV molecular testing.[13] The NS1 antigen test, with high specificity (100%) and moderate sensitivity (34% to 76%), is utilized as a first diagnostic option in primary care.[11] The detection of the NS1 DENV antigen is crucial for DVI diagnosis, particularly early in the course of the disease. The NS1 antigen, a glycoprotein crucial to DENV survival,[14] can be detected using various

Cite this article: Sunari IGAAEP, Aryati A, Hakim FKN, Tanzilia MF, Parwati I, Yaswir R, Mulyono B. Positive NS1 Antigen in Non-Dengue Virus Infection Serum: Possible Reasons for the Discrepancy with DENV PCR Results. Pharmacogn J. 2024;16(4): 923-926.

techniques, such as immunochromatography (ICT), fluorescence immunoassay (FIA), and enzyme-linked immunosorbent assay (ELISA). Each technique has its benefits, drawbacks, and diagnostic values.[15] Pal (2014) reported higher sensitivity for NS1 antigen detection using the ELISA method compared to ICT.[15] Similarly, a study conducted in Malaysia by Chua et al. found that the NS1 antigen examination using the ELISA method was more sensitive than virus isolation and real-time RT-PCR.[16]

In this study, researchers aim to analyze the presence of NS1 antigen positivity in non-DVI samples confirmed by real-time RT-PCR examination with semi-quantitative NS1 antigen examination. The results are expected to provide insight into the advantages and disadvantages of laboratory examination modalities for detecting dengue virus pathogens.

METHODS

Patients

This study was part of a larger investigation exploring the epidemiology of dengue. The study population consisted of 130 patients with confirmed non-DVI by RT-PCR examination. These patients, aged 1-65 years, presented with acute febrile illness and attended primary healthcare centers in Jember, Tulungagung, and Surabaya from March 2019 to February 2020. Acute febrile illness was defined as an axillary temperature > 38 °C lasting less than 5 days. Serum samples were initially stored at -20 °C at the study sites and then transferred weekly to Dr. Soetomo General Academic Hospital in Surabaya, where they were stored at -70 °C.

Serum Sample Selection

Patients with an insufficient serum volume or incompletely filled-out case record forms were excluded from the study.

NS1 Antigen ELISA

All serum samples were tested for dengue NS1 antigen (NS1Ag) using the Platelia™ Dengue NS1Ag-ELISA (Bio-Rad Laboratories). This one-step enzyme-linked immunoassay (ELISA) qualitatively or semi-quantitatively detects dengue virus NS1 antigen. The kit employs a sandwich enzyme microplate immunoassay with murine monoclonal antibody peroxidase as the probe. The procedure followed the manufacturer's instructions. The examination results were expressed as an S/CO ratio, where S is the optical density of the sample, and CO is the average calibration repetition. Interpretation of the ratio values was categorized as positive, negative, or equivocal.

DENV Polymerase Chain Reaction

For nucleic acid extraction, 140 µL of serum was used with the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). Collected RNA was stored at -70 °C before performing real-time RT-PCR according to the manufacturer's protocols. DENV serotype detection was conducted using real-time RT-PCR with the Dengue RT-PCR Test Real-Time instrument.

Data analysis

Statistical analyses were performed using SPSS for Windows (version 16.0.1; SPSS, Chicago, IL). Frequencies and means or medians were calculated to describe background variables. Predictive values were determined by cross-tabulating the true diagnosis versus NS1Ag-based diagnoses.

RESULTS

The study utilized 130 stored non-DVI samples, which were confirmed to be free of dengue virus through real-time RT-PCR. All samples were tested for NS1 antigen using ELISA. Of the 130 samples, 4 were positive for NS1Ag. The examination process is illustrated in Diagram 1, and the demographic results are presented in Table 1.

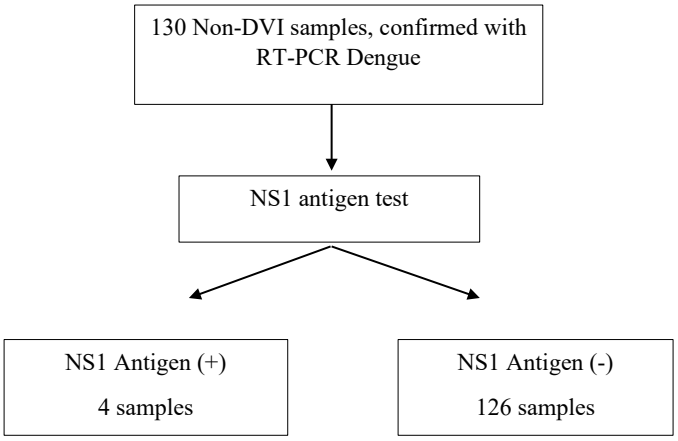

Diagram 1. Flow diagram of the identified studies

The demographic data showed an average age of 19 years, with 52% of the samples being male. The average duration of fever was 3 days. According to the NS1 Ag ELISA results, 4 (3.3%) of the DENV-negative samples tested positive for NS1Ag.

All four samples that tested positive for NS1Ag were male and developed a fever on the fourth day. The optical density ratios (ODRs) for these samples were found to be greater than 1. The positive samples included both adult and pediatric patients, with two samples from each group. In total, 3.08% of the samples were NS1Ag positive.

DISCUSSION

Molecular examination remains the gold standard for virus detection, with DENV RT-PCR being one of the primary methods for diagnosing Dengue virus infection.[17] DENV RT-PCR boasts 100% specificity but has varying sensitivity between 28.8% and 76.6%(10). Variations in DENV PCR sensitivity can lead to false-negative results, which is a significant concern as it affects the management of DVI therapy. The NS1 antigen test is still favored for early diagnosis of dengue virus infection due to its high specificity (100%).[18] In this study, the population consisted of stored biological samples from fever patients confirmed as non-DVI using real-time RT-PCR.

Among the 130 negative DENV RT-PCR samples, 4 (3.08%) tested positive for the NS1 antigen. The discrepancy between the RT-PCR and

Table 1. Demographic data of enrolled patients.

Demographic	Result
Age	19 (1-60) years old
Sex	
Male	68 (52.3 %)
Female	62 (47.7 %)
Day of Fever	3 (1-4) days
NS1 Ag ELISA	
Positive	4 (3.1 %)
Negative	126 (96.9 %)

Table 2. Characteristics of 4 non-DVI samples with positive NS1 Ag.

No Sample	Days of fever	Ages (y.o)	Gender (M/F)	ODR value
092	4	26	M	7.53
104	4	8	M	8.02
106	4	15	M	5.17
110	4	27	M	7.72

NS1 antigen results can be influenced by several factors. A negative DENV RT-PCR result could indicate either a true negative or a false negative. Researchers believe this discrepancy may be due to the varying sensitivity of DENV PCR. Factors influencing DENV PCR sensitivity include the type of PCR method used, primers, the presence of DENV mutations, DENV serotypes, and mismatched nucleotides. Aryati (2014) noted that conventional PCR methods have lower sensitivity compared to real-time PCR methods.[10,19] This study, however, does not discuss the specific DENV PCR primers used or the possibility of DENV mutations, necessitating further research.

The specificity of the NS1 antigen in this study was slightly lower than the 100% specificity found in the study by Aryati et al. (2013) in the non-DVI group and healthy patients(18). Other studies reported NS1 antigen sensitivity and specificity values of 93.4% and 100%, respectively, in primary infection, with 100% PPV and 97.3% NPV.[20] Kumarasamy (2007) also found that NS1 antigen-capture ELISA had higher sensitivity and specificity than virus isolation and RT-PCR in the early stages of laboratory confirmation for single serum samples of dengue infection patients, particularly in cases of primary infection. The diagnostic value improves when combined with IgM capture-ELISA.[16]

Gaikwad (2017) reported sensitivity, specificity, PPV, and NPV values of 89.9%, 100%, 100%, and 94%, respectively, for 200 serum samples from patients suspected of having dengue. These results are better than those of the rapid dengue NS1 antigen, which had values of 81.5%, 66.7%, 78.2%, and 71.1%. In the early stages of dengue, especially during epidemics, NS1 ELISA is very beneficial. However, the requirement for sophisticated instruments and resources limits the routine use of ELISA as a first diagnostic technique for dengue infection patients.[21,22]

Considering the high specificity of the NS1 antigen, the 3.08% discrepancy between DENV PCR and NS1 antigen results might be attributed to the low detection ability of DENV PCR due to the factors mentioned above. Each method has its advantages and disadvantages, and this study did not aim to compare the assessment of the PCR method and the NS1 antigen. Dengue RT-PCR data must be interpreted with caution. Despite its high specificity and variable sensitivity, false-negative results can occur, potentially impacting DVI management.

CONCLUSION

Researchers believe that the varying sensitivity of DENV PCR could explain the discrepancies observed in this study. Factors such as the type of PCR technology, the primers used, the presence or absence of DENV mutations, DENV serotypes, and mismatched nucleotides can all influence DENV PCR sensitivity. Therefore, accuracy is crucial when interpreting DENV RT-PCR test findings, as several factors can lead to false-negative results.

ACKNOWLEDGMENTS

The authors would like to thank the Universitas Airlangga Surabaya (965/UN3.14/LT/2019).

ETHICAL CONSIDERATIONS

The study was initiated after obtaining ethical approval from the Research Ethics Committee of the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia (Ethical Approval No. 156/EC/KEPK/FKUA/2019, 17 May 2019). The voluntary involvement in the study was ensured by obtaining written informed consent from all patients and/or children's legal guardians.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Beltrán-Silva SL, Chacón-Hernández SS, Moreno-Palacios E and Pereyra-Molina JÁ: Clinical and differential diagnosis: Dengue, chikungunya and Zika. *Revista Médica del Hospital General de México*. 2018;81:146–153.
2. Indonesian Ministry of Health: Action Plan for Vector-Borne and Zoonotic Disease Prevention and Control Activities 2015-2019. Ministry of Health of the Republic of Indonesia. 2017;1–58.
3. Sudaryanto A, Ainnurriza US, Supratman S, Dewi SK. Mapping the prevalence of dengue fever in Sragen Regency Indonesia. *Bali Medical Journal*. 2021;10:1107–1110, 2021.
4. Pinontoan OR, Sumampouw OJ, Ticoalu J, Nelwan JE, Musa EC and Sekeeon J: The variability of temperature, rainfall, humidity and prevalence of dengue fever in Manado City. *Bali Medical Journal*. 2022;11:81–86.
5. Setiawan AF, Prabowowati Wajib YY, Handono K, Sakti SY. Leucocytes, thrombocytes, and immature platelets in patients with dengue hemorrhagic fever. *Bali Medical Journal*. 2023;12:2067–2069.
6. Gasem MH, Kosasih H, Tjitra E. Correction: An observational prospective cohort study of the epidemiology of hospitalized patients with acute febrile illness in Indonesia. *PLOS Neglected Tropical Diseases*. 2022;16:e0010530,.
7. Utama IMS, Lukman N, Sukmawati DD. Dengue viral infection in Indonesia : Epidemiology, diagnostic challenges, and mutations from an observational cohort study. *PLoS Neglected Tropical Diseases*. 2019;19:1–19.
8. Yantie NPVK, Gunawijaya E, Suradipa IW, Gustawan IW. Asymptomatic Cardiac Rhythm Abnormality in Children with Dengue Virus Infection. *Bali Medical Journal*. 2016;5:177.
9. Tanzilia M, Zuroidah N, Sunari IGAAEP. Comparative Diagnostic Value of Anti-Dengue IgG, Anti-Dengue IgM of Two Rapid Tests in Dengue Virus Infection. *International Journal of Pharmaceutical Research*. 2022:14.
10. Sasmono RT, Aryati A, Wardhani P. Performance of Simplexa Dengue Molecular Assay Compared to Conventional and SYBR Green RT-PCR for Detection of Dengue Infection in Indonesia. *PLoS ONE*. 2014;9:e103815.
11. Tang KF and Ooi EE: Diagnosis of dengue: an update. E*xpert Review of Anti-infective Therapy*. 2012;10:895–907.
12. Peeling RW, Artsob H, Pelegrino JL. Evaluation of diagnostic tests: dengue. *Nature Reviews Microbiology*. 2010;8:S30–S37.
13. Yow KS, Aik J, Tan EYM, Ng LC, Lai YL. Rapid diagnostic tests for the detection of recent dengue infections: An evaluation of six kits on clinical specimens. *PLoS ONE* 16: 1–11, 2021.
14. Baihaki I, Dewi BE, Kharisma VD. Correlation of the Presence of Non Structural-1 (NS1) Antigen Dengue Virus with Severity of Dengue Infection. *Pharmacognosy Journal*. 2023;14:813–816.
15. Pal S, Dauner AL, Mitra I. Evaluation of Dengue NS1 Antigen Rapid Tests and ELISA Kits Using Clinical Samples. *PLoS ONE*. 2014;9:e113411.
16. Chua KB, Mustafa B, Abdul Wahab AH. A comparative evaluation of dengue diagnostic tests based on single-acute serum samples for laboratory confirmation of acute dengue. *The Malaysian Journal of Pathology*. 2011;33:13–20.
17. Shu P-Y, Huang J-H. Current Advances in Dengue Diagnosis. *Clinical and Vaccine Immunology*. 2004;11:642–650.
18. Aryati A, Trimarsanto H, Yohan B, Wardhani P, Fahri S, and Sasmono RT: Performance of commercial dengue NS1 ELISA and molecular analysis of NS1 gene of dengue viruses obtained during surveillance in Indonesia. *BMC Infectious Diseases*. 2013;13: 611.

19. Chien LJ, Liao TL, Shu PY, Huang JH, Gubler DJ, Chang GJJ. Development of real-time reverse transcriptase PCR assays to detect and serotype dengue viruses. *Journal of Clinical Microbiology*. 2006;44:1295–1304.

20. Lapphra K, Sangcharaswichai A, Chokephaibulkit K, *et al.*: Evaluation of an NS1 antigen detection for diagnosis of acute dengue infection in patients with acute febrile illness. *Diagnostic Microbiology and Infectious Disease.* 2008;60: 387–391.

21. Kumarasamy V, Chua SK, Hassan Z, Wahab AHA, Chem YK, Mohamad M, and Chua KB: Evaluating the sensitivity of a commercial dengue NS1 antigen-capture ELISA for early diagnosis of acute dengue virus infection. *Singapore Medical Journal*. 2007;48: 669–73.

22. Gaikwad S, Sawant SS, Shastri JS. Comparison of non-structural protein-1 antigen detection by rapid and enzyme-linked immunosorbent assay test and its correlation with polymerase chain reaction for early diagnosis of dengue. *Journal of Laboratory Physicians*. 2017;9:177–181.

Cite this article: Sunari IGAAEP, Aryati A, Hakim FKN, Tanzilia MF, Parwati I, Yaswir R, Mulyono B. Positive NS1 Antigen in Non-Dengue Virus Infection Serum: Possible Reasons for the Discrepancy with DENV PCR Results. Pharmacogn J. 2024;16(4): 923-926.