Innovative Journal of Medical and Health Science

IJMHS 10 (12), 1425–1431 (2020) ISSN (O) 2277:4939 | (P) 2589:9341

SENSITIVITY VALUE OF IgA AND NS1 SEROLOGIC TEST IN EARLY DIAGNOSIS OF DENGUE INFECTION IN FEBRIS PATIENTS 3-5 DAYS, WHICH ONE IS MORE EFFECTIVE? (A Case study in Cimahi, West Java, Indonesia)

Yudith Yunia Kusmala[1], Rini Sundari[1], Tezza Adriansyah Anwar[*,†,1], Jovy Yudha Tamba[2]

[1] *Faculty of Medicine, Universitas Jenderal Achmad Yani*
[2] *Medical Study Program, Faculty of Medicine, Universitas Jenderal Achmad Yani*

DOI: https://doi.org/10.15520/ijmhs.v10i12.3164

Accepted 07/11/2020; Received 15/10/2020; Publish Online 07/12/2020

ABSTRACT

The aim of this study was to determine the sensitivity level of IgA and NS1 serological tests in early diagnosis of dengue infection in febris patients 3–5 days in Cimahi. Next is to find out which of the two serological tests is the most effective for early diagnosis of dengue infection in febris patients for 3-5 days. The incidence and death rate due to dengue virus infection is still quite high, especially in several areas in Indonesia. The clinical manifestations of dengue virus infection are very diverse and the outcome of dengue infection (prognosis) is difficult to predict. An efficient and accurate diagnosis of dengue is the most important part of the patient care process. Serological examination is a laboratory diagnostic method that is often used to confirm the presence of dengue virus infection. NS1 and IgA anti-dengue are serologic tests that are often used, especially in the acute phase of the disease. This study aims to determine differences in the sensitivity of NS1 and IgA in patients with dengue infection with a fever of 3–5 days. This study was a prospective analytic observational study with a cross-sectional design. The subjects of this study were patients with dengue infection with a duration of 3-5 days. The number of samples that met the inclusion criteria was 69 people. The results of this study showed differences in the sensitivity of NS1 and IgA to antidengue in patients with dengue infection with a fever of 3-5 days. NS1 examination had the highest sensitivity value on the 3rd day of 75.0% and the lowest on the 5th day of 52.4%. Anti-dengue IgA examination had the lowest sensitivity value on the 3rd day of 10.0% and the highest on the 5th day of 42.9%.

Key words: S1–IgA Antidengue–Dengue infection–sensitivity

1 INTRODUCTION

Dengue infection is a disease that is found in most tropical and subtropical regions, especially Southeast Asia, Central America, America, and the Caribbean.3 Indonesia is one of the countries that has experienced outbreaks of dengue infection. Based on epidemiological data nationwide, in 2015 the incidence of dengue fever in Indonesia increased again as many as 126,675 dengue hemorrhagic fever sufferers with a death rate reaching 1,299 people.4,5

West Java is one of the provinces that has a fairly high number of dengue fever cases in 2015, reaching 22,111 cases higher than 2014 (19,138 cases), with the number of deaths reaching 184 people with a CFR of 0.83% in 2015. In 2016, the incidence of dengue fever continued to increase as many

* Corresponding author.
† Email: tezza_anwar@yahoo.com

as 37,418 cases, higher than in 2015 with the death rate reaching 277 people.6

Meanwhile, Cimahi is one of the cities in West Java Province that has a fairly high incidence of dengue fever. In 2015 the number of cases reported was 797 cases with IR 135 / 100,000 population and CFR 0.63%. In 2014, there were 514 cases reported, with an lR 92.09/ 100,000 population and a CFR of 0 (no deaths due to dengue hemorrhagic fever were found). Overall, from 2015 there was an increase in the number of cases when compared to 2014.7-9

The diagnosis of dengue hemorrhagic fever is confirmed using the criteria of the World Health Organization (WHO) in 1997, namely clinical criteria in the form of sudden and continuous high fever for 2−7 days, bleeding manifestations, either by a positive tourniquet test or in other forms such as ptekie, epistaxis, bleeding gums, hematemesis, melena, and hepatomegaly.2,10,11 Laboratory criteria obtained thrombocytopenia less than 100,000/μl or an increase in hematocrit of 20% or more.3,11 The clinical picture caused by dengue virus infection is often atypical and average. average appeared on day 3−7.2,3

The clinical symptoms of dengue infection often resemble other diseases, such as influenza, typhoid fever, chikungunya fever, leptospirosis, malaria, and various other infections. Routine laboratory tests (thrombocytes and hematocrit) do not do much to help diagnose this disease precisely, because the resulting laboratory images are sometimes the same between dengue fever, dengue hemorrhagic fever, and dengue shock syndrome.12,13 A quick and precise diagnosis is needed to differentiate the situation. dengue fever, dengue hemorrhagic fever, and dengue shock syndrome, especially on the first day of the onset of the disease so that action can be determined quickly and precisely.1,13,14

Delay in diagnosis is still one of the problems in cases of dengue hemorrhagic fever which causes a high mortality rate due to complications caused by the disease. disease to a more severe direction, so that the death rate due to dengue fever can continue to decline until it is not found.5,15

The more difficult the early detection of dengue virus infection based on signs and symptoms, laboratory examination is one of the most important supporting tests to establish a diagnosis.14,16 Laboratory tests on dengue fever are divided into two parts, namely specific and non-specific investigations. To make a diagnosis of dengue fever, specific investigations are needed. The supporting examination consists of virus isolation, genome examination and antigen detection, and serological examination. Until now, serological examination is still the main choice for diagnosing dengue hemorrhagic fever. Serological diagnosis of dengue virus infection, which is currently the WHO standard, is the hemagglutination barrier test. This method can determine primary infection or secondary infection. It is sensitive but not specific because it can detect infections by other Flavivirus families. Previous serological tests such as the complement fixation test and neutralization test are no longer used because they are less sensitive, impractical, expensive, require a long processing time, and are difficult techniques. Currently, the choice is antidengue serologic examination using immunochromatography IgG and IgM methods. This serological examination has good accuracy with a sensitivity value of 97.7% and a specificity of 92.5%, the results are fast, easy to do, and the cost is relatively cheap compared to other tests. This serological examination could not detect the dengue virus early, because IgM antibodies that show an infection will often appear on the 5th day after infection or fever symptoms appear.17-19

At present there are several breakthroughs in new examinations to support early diagnosis of cases of dengue hemorrhagic fever, one of which is the nonstructural antigen 1 dengue (NS1). The NS1 antigen is a glycoprotein measuring 46-50 kilodaltons and produced by all dengue virus serotypes. NS1 antigen is found in secreted and non-secreted forms with high levels in patients with acute dengue virus infection. NS1 antigen can be detected in serum or plasma on days 1–9 of fever onset. The NS1 antigen has been in circulation during the viremia phase of dengue virus infection or when the dengue virus replicates in the human body. The highest level of NS1 was obtained on day 3 of fever with sensitivity values on days 1–4 reaching 87.6% with a specificity of 100%, while for days 5−10 fever the sensitivity value of NS1 decreased to 52.2%, but specificity remained. 100%.20

In recent years, immunoglobulin A (IgA) has become one of the parameters in the early diagnosis of acute dengue hemorrhagic fever infection. Immunoglobulin A is a glycoprotein molecule in the form of monomers or dimers and is produced by plasma cells in response to immunogens and functions as an antibody. IgA can be detected in the blood on days 1-7 when the patient has fever, high levels of antidengue IgA on the first day of fever are due to involvement of the mucosa-associated lyhmphoid tissues (MALT) in dengue fever infection. In acute infection, when the virus enters the human body, the dengue virus multiplies in antigen presenting cells (APC) and stimulates local immune cells to activate plasma cells and produce anti-dengue IgA which will enter the circulation. Based on research conducted by Aryati, et.al. It was found that the immunoglobulin A could be detected from the first day when the patient had a fever with a sensitivity value of 83.3% and a specificity of 81.1% .22 The highest sensitivity value was obtained on the first to the third day of 94.7%, and decreased on day 1. fourth to fifth to 77.7% and decreasing on the sixth to seventh days to 64.3%.12

Based on the description above, the two investigations, both NS1 and IgA, have great potential in making an early diagnosis of dengue fever. Therefore, this study aims to determine the sensitivity level of IgA and NS1 serological tests in early diagnosis of dengue infection in febris patients 3–5 days in Cimahi. Next is to find out which of the two serological tests is the most effective for early diagnosis of dengue infection in febris patients for 3-5 days.

2 LITERATURE REVIEW

a. The laboratory diagnosis of dengue infection can be confirmed by detecting specific viruses, genome sequences,

antibodies, and viral antigens. Virus culture or PCR is currently considered the gold standard for detecting dengue virus, however, it has limitations in terms of cost, time, and technical processing. Serologic examination is one of the examinations that are often performed by clinicians to confirm the diagnosis of dengue fever. Serological diagnosis of dengue virus infection, which is currently the WHO standard, is the hemagglutination barrier test. This method can determine primary infection or secondary infection. It is sensitive but not specific because it can detect infections by other Flavivirus families. Previous serological tests such as the complement fixation test and neutralization test are no longer used because they are less sensitive, impractical, expensive, require a long processing time, and are difficult techniques. Currently the choice is antidengue serologic examination using the IgG and IgM immunochromatography methods.2,18,19

NS1 examination can be done on the first day to the 8th day since the onset of fever. Immunoserological examination is performed by checking IgM and IgG against dengue. IgM is detected from day 3-5, increases to week 3 and disappears after 60-90 days. Positive IgM results usually indicate primary dengue infection. IgG was detected on day 11 in primary infection, and day 2 in secondary infection.17-19

Currently, IgA antibody testing is used as a method for diagnosing dengue infection. IgA is the main secretory antibody and human serum contains very low concentrations of IgA.

In dengue infection, there is an increase in the value of IgA antibodies in human serum.30

b. NS1 Examination

At present, a test for dengue non-structural antigen-1 (NS1) has been developed which can detect dengue virus infection early even on the first day of fever. The NS1 protein in the dengue virus is a glycoprotein measuring 46–50 kilodaltons which is expressed in both membrane associated (mNS1) and secreted (sNS1) infected host cells and is not a part of the virion structural component. The dengue virus has an 11 kb genome that encodes 10 kinds of viral proteins, namely three structural proteins (C / core protein, M / membrane protein, E / envelope protein) and seven nonstructural proteins (NS-1, NS-2a, NS-2b, NS.

-3, NS-4a, NS-4b, NS-5).26

NS1 protein is produced by all flavivirus and plays an important role in the process of virus replication and survival. The NS1-antibody complex will activate the complement system which in turn causes microvascular leakage. NS1 is a toll-receptor 4 agonist that stimulates myeloid cells to produce various cytokines that contribute to the severity of dengue infection, such as IL-10. In addition, NS1 can directly damage endothelial cells leading to plasma leakage.1,27,28,29

When the virus enters cells through the process of endocytosis through receptors, the viral genome consisting of single-chain RNA will be released into the cytoplasm and used as a template for the translation process to become larger protein precursors. Dengue virus infected cells express NS1 protein on the cell surface so that NS1 protein is found in the patient's serum in the early phase. When the dengue virus binds to host cells, the virus enters through endocytosis. Endosomes induce fusion. The viral nucleocapsid will be released so that the virus becomes uncoated. Viral polyproteins are synthesized when the virus combines with the endoplasmic reticulum to form 3 structural proteins and 7 non- structural proteins. Furthermore, the virus changes from translation to vRNA through asymmetric synthesis. Translation produces large amounts of protein. The virions are assembled in the endoplasmic reticulum and pass through the Golgi apparatus and then the virions mature in the Golgi apparatus. The NS1 protein that has been expressed is cut by viral enzymes and host cell protease enzymes in the endoplasmic reticulum and secreted on the plasma membrane, so that this protein can be used as a marker for dengue hemorrhagic fever.

The use of NS1 examination in diagnosing dengue has been suggested, especially in the early phase since the onset of fever. Several studies have revealed that dengue NS1 is a very important biomarker in the diagnosis of dengue infection. NS1 antigen can be detected from the first day since the onset of fever and reaches its peak on the third to fifth day.29 Currently, various methods have been developed to detect NSI antigens, including

antigen-capture ELISA, lateral flow antigen detection, and rapid diagnostic test using commercial kit. The sensitivity of NS1 examination ranged from 63-93.4% with a specificity of 100% as high as the specificity of the gold standard viral culture. However, a negative NS1 result does not exclude the presence of dengue virus infection.5,22,29,30

c. Anti-dengue IgA Examination

The antibody response to infection includes the appearance of various types of immunoglobulins. Immunoglobulin was the first substance to be identified as a molecule in serum capable of neutralizing a number of infectious microorganisms. This molecule is formed by B cells in 2 different forms, namely surface receptors for antigens and as antibodies secreted into extracellular pathways. The basic structure of immunoglobulin consists of 2 identical heavy chains (H-chains) and 2 identical light chains (L-chains). Immunoglobulin consists of 3 fragments, namely 2 fragments that have the same arrangement consisting of heavy chains (H) and light chains (L) called Fab fragments formed by N-terminal domains, and 1 fragment consisting only of heavy chains is called fragment Fc formed by the C-terminal domain.

The Fab fragment with the antigen binding site functions to bind the antigen, because the amino acid arrangement in this section differs from one immunoglobulin molecule to another and is highly variable according to the variability of the antigen that stimulates its formation. On the other hand, fragment Fc is a constant fragment. This fragment does not have the ability to bind to antigens but can act as an antigen (antigen determinant). This fragment has a secondary effector function and determines the biological characteristics of the immunoglobulin in question, for example the ability of immunoglobulins to attach to cells, complement fixation, ability of Ig to penetrate the placenta, distribution of immunoglobulins in the body and others.

Immunoglobulin A has a molecular weight of 165,000 daltons and is found in serum in small amounts, but its levels in the secretions of the respiratory tract, gastrointestinal tract, urinary tract, tears, sweat, saliva and breast milk are higher in the form of secretory IgA. The half-life of IgA is six days and the active form is the dimer, while the monomer is inactive. The tissues that secrete dimer forms are epithelial cells that act as IgA receptors, which then these cells with IgA enter the lumen.

Immunoglobulin A is the first antibody to appear in cases of secondary dengue infection before IgG and IgM are produced. Meanwhile, in the case of primary infection, IgA is produced after the emergence of anti-dengue IgM. Based on the serological pattern of secondary dengue infection, it can be seen that the emergence of anti-dengue IgA is high in the early phase of fever until the seventh day after the onset of fever, while the first anti- dengue IgM is detected on the fifth day after the onset of fever. The increase in anti-dengue IgA on the first day of fever was due to involvement of mucosa-associated lymphoid tissue (MALT). Dengue virus multiplies in APC and stimulates local immune cells to activate plasma cells and produce anti-dengue IgA which will enter the circulation through high endothelial venules (HEV).22,31,32,33

Based on research conducted by Aryati et al. It was found that immunoglobulin A could be detected since the first day when the patient had a fever with a sensitivity value of 83.3% and a specificity of 81.1%. This is also supported by previous research conducted by Yun Ying Tan et al., That examination of anti-dengue IgA has a sensitivity range ranging from 84.80-86.70% and a varying specificity of 80-92%.12

3 METHODOLOGY

The subjects in this study were dengue infection patients with a fever of 3–5 days who came to the Dustira Hospital, Mitra Kasih Hospital, and the Kasih Bunda Hospital in Cimahi from December 2018 to January 2019. The NS1 and IgA anti-dengue examinations were carried out at Clinical Pathology Laboratory, Faculty of Medicine, Unjani.

Consecutive sampling was used in this study. Samples were febrile patients 3–5 days who met the inclusion and exclusion criteria. By using the formula for determining the sample size for diagnostic test research with sensitivity output, the minimum total sample size of the research sample is at least 59 people.35,36

The research material used in this study was serum of febrile patients 3-5 days from the onset of fever in the hospital. Serum is certainly not hemolyzed, lipemic, icteric, and contaminated with particles or bacteria because it can affect the test results. Meanwhile, the tools used in this study were the MP diagnostics MULTISURE → dengue Ab / Ag NS1 rapid test, and anti-dengue IgA, syringes, tourniquets, disposable pipettes, test tubes without anticoagulants, centrifuges, and medical records for febrile patients 3–5 days at Dustira Hospital, Mitra Kasih Hospital, and Kasih Bunda Hospital.

The normality test of the research data was carried out using the Shapiro-Wilk test. The significance test to compare the characteristics of the two research groups used an unpaired t test if the data were normally distributed, and the Mann Whitney test as an alternative if the data were not normally distributed. Meanwhile, statistical analysis for

categorical data was tested with the chi-square test if the Chi-Square requirements were met, if not met, then the Exact Fisher test was used for 2 x 2 tables and Kolmogorov Smirnov for tables other than 2 x 2.34,35,36.

4 RESULT AND DISCUSSION

Based on table 1., NS1 was one of the more reactive tests than anti-dengue IgA, especially on the third and fourth days. Meanwhile, on day 5, reactive NS1 examination decreased compared to days three and four. On the 5th day of examination for NS1, there was a significant decrease in reactivity, only 11 of 21 people who had dengue fever or 52.4% were reactive NS1.38,39

According to research conducted by Mulyono, et.al. the pattern of serological examination in dengue infection begins with the emergence of NS1 antigen on the first day of fever and continues to increase until it reaches a peak on days 3–5 of fever onset. It then decreases to undetectable levels 5–6 days after the onset of fever. This is thought to be related to the pattern of dengue virus infection in the human body.12 When the virus enters cells through the endocytosis process through receptors, the viral genome consisting of single-chain RNA is released into the cytoplasm and used as a template for the translation process to become

protein precursors.25,42, Dengue virus-infected cells express NS1 protein on the cell surface so that NS1 protein is found in the patient's serum, especially in the early phase of fever.26

The results of this study are in accordance with the research conducted by Dwiyanti, et.al., where the mean high NS1 antigen levels at the onset of illness, the highest on the 2nd sick day, decreased before and simultaneously with the defervescence phase.37 Average NS1 antigen levels high at the beginning of illness, both in dengue fever and dengue hemorrhagic fever, then decreased.20,29,37 NS1 antigen showed a decrease in reactive value with increasing sick days. This is in accordance with the results of research that has been carried out at this time.29,37

From the research results also obtained differences in the sensitivity values of the examination using NS1 and IgA as follows:

Based on Table 2. NS1 examination had the highest sensitivity value on the 3rd day and the lowest on the 5th day of fever onset. This is because the dengue virus has two types of protein, namely structural protein and non-structural protein. Structural protein consists of protein E, protein M, and protein C, while non-structural protein consists of seven proteins NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5.23,24 NS1 protein will be expressed on the surface of the host cell when viruses dengue infects the human body.

Table 1. NS1 and IgA Examination Results based on Fever duration

DESCRIPTIONS	FEVER DURATIONS 3 Days		4 Days		5 Days	
	Q	%	Q	%	Q	%
NS1 Examination Result						
Reactive	15	75,0%	18	66,7%	11	52,4%
Non- Reactive	5	25,0%	9	33,3%	10	47,6%
TOTAL	20	100,0%	27	100,0%	21	100,0%
IgA Examination Result						
Positive	2	10,0%	6	22,2%	9	42,9%
Negative	18	90,0%	21	77,8%	12	57,1%
TOTAL	20	100,0%	27	100,0%	21	100,0%

Table 2. Sensitivity Values for NS1 and IgA Tests Based on Fever Durations

SENSITIVITY VALUE	FEVER DURATIONs 3 Days	4 Days	5 Days
NS1	75,0%	66,7%	52,4%
IgA anti dengue	10,0%	22,2%	42,9%

The NS1 protein that has been expressed is cut by viral enzymes and host cell protease enzymes in the endoplasmic reticulum and secreted on the plasma membrane, so that this protein can be used as a marker of dengue infection, especially in the acute phase.29,30

The results of this study are in accordance with research previously conducted by Dussart, et.al. where the NS1 examination is one of the tests that can be done from day 1–9 of the onset of fever.22 NS1 antigen can be detected from the first day since the onset of fever and reaches its peak, especially on the third to fifth day of fever onset. The NS1 antigen has been in circulation during the viremia phase of dengue virus infection or when the dengue virus replicates in the human body. In that study, the highest level of NS1 was found on day 3 of fever with sensitivity values on days 1–4 reaching 87.6%, while for days 5–10 fever the sensitivity value of NS1 decreased to 52.2%. The results of other studies conducted by Badave, et.al., showed that NS1 examination had a good diagnostic value in the acute phase of the disease, namely 73.53%, this result was better than the diagnostic value of platelet counts, leukocytes and antibodies to IgG and IgM antidengue.22

In this study, the average sample was dominated by primary dengue infection patients compared to secondary dengue infection. The results of this study are also in accordance with the study conducted by Kumarasany, et.al., who obtained high NS1 antigen sensitivity (93.4%) had a larger primary infection sample (86.4%), with greater NS1 antigen positivity (97.3%) in primary infection than secondary infection.64 Other studies also found that Ag NS1 was detected more in primary infection than secondary infection with dengue. This is also similar to the study conducted by Tricou et al. obtained Ag NS1 positivity of 61.6%, with a smaller number of samples of primary infection (26.9%) than secondary (71.8%), and higher positivity for primary dengue infection (80.3%) than secondary (55, 1%). According to Tricou, secondary infection was one of the factors that caused the NS1 antigen to be found negative in the sample.

Meanwhile, from Table 2, it was also found that the IgA sensitivity value for anti- dengue had a significant increase starting on day 3 and had the highest value on day 5 of fever onset. This is thought to be because anti-dengue IgA appears at the same time as anti- dengue IgM in patients with primary dengue infection. The results of the study conducted by the researchers, the majority of the study subjects were diagnosed with primary dengue infection compared to secondary dengue infection. This result is similar to the research conducted by Djatnika, et.al. who got the number of research subjects for primary dengue infection were 15 patients and as many as 11 people with secondary dengue infection.41 In contrast to the results of research conducted by Aryati, et.al. where on average the majority of subjects in the study were diagnosed with secondary dengue infection compared to primary dengue infection.12 In primary infections the percentage of positive anti-dengue IgA was 100%, while anti-dengue IgM was only 50% and NS1 did not show positive results. On day 3-4, the proportion of anti-dengue IgA was the same as anti-dengue IgM. On day 5-6, the positive results for anti-dengue IgA were higher than those for anti-dengue IgM or NS1. The percentage of positive anti-dengue IgA in secondary infections was higher than anti-dengue IgM or NS1, but almost the same as anti-dengue IgG.32,41

Talarmin, et.al. reported that anti-dengue IgA increased at the same time as anti- dengue IgM in patients with primary dengue infection. Anti-dengue IgA occurs on day 5-6 in primary dengue infection.40 In secondary dengue infection, immunoglobulin A appears earlier than anti-dengue IgG and IgM. In secondary dengue infection, there is stimulation of the immune system to produce high levels of anti-dengue IgA through rapid seroconversion of memory cells from previous infections.40 Therefore, in secondary dengue infection, IgA anti-dengue is higher than anti-dengue IgM or NS1. Based on the serological pattern of secondary dengue infection, it can be seen that the appearance of anti-dengue IgA is high in the early phase of fever until the seventh day

after the onset of fever, while the first anti- dengue IgM is detected on the fifth day after the onset of fever.

Based on research conducted by Aryati, et.al. It was also found that in secondary infection, immunoglobulin A could be detected since the first day when the patient had a fever with a sensitivity value of 83.3% and a specificity of 81.1%. This is also supported by previous research conducted by Yun Ying Tan et al. that the anti-dengue IgA examination had a sensitivity range ranging from 84.80 to 86.70%.40

5 CONCUSSION

There are differences in the sensitivity value of NS1 and IgA to anti-dengue in patients with dengue infection with fever duration between 3–5 days. NS1 examination had the highest sensitivity value on day 3 (75.0%) and the lowest on day 5 (52.4%). Meanwhile, anti-dengue IgA examination had the lowest sensitivity value on day 3 (10.0%) and the highest on day 5 (42.9%). NS1 antigen examination is the most reactive test in patients with dengue infection with a fever of 3–5 days.

In suggesting dengue serology examination, practitioners need to pay attention to the duration of fever to suggest the appropriate type of examination to confirm the diagnosis. Serologic examination of NS1 antigen can be requested by clinicians, especially in patients with primary dengue infection with fever 3–5 days. Future studies can be conducted to compare the sensitivity value of NS1 and IgA to antidengue in patients with secondary dengue infection.

6 RESEARCH LIMITATIONS

In this study there are several limitations:

- The degree of certainty of the duration of fever suffered by dengue infection patients.
- Researchers ran out of time when making informed consent to each patient, because of the high rate of patient anxiety to participate as research subjects.
- Limitations of the volume of blood provided by the hospital laboratory.
- The limited time for the study was due to the difficulty of licensing each hospital.

1. Wowor, MF. 2011. Deteksi dini demam berdarah dengue dengan pemeriksaan antigen NS1, Fakultas Kedokteran Universitas Sam Ratulangi.
2. World Health Organization. 2012. Handbook for clinical management of dengue, WHO.Geneva.
3. Candra A. 2010. Epidemiologi , patogenesis , dan faktor risiko penularan, Aspirator 2010; 110-9.
4. Kementerian Kesehatan Republik Indonesia. 2016. Situasi DBD di Indonesia. Info DATIN, Jakarta.
5. Kementerian Kesehatan Republik Indonesia, 2010. Demam berdarah dengue. Kemenkes Jakarta.
6. Dinas Kesehatan Provinsi Jawa Barat. 2016. Profil kesehatan Provinsi Jawa Barat tahun 2015. Dinkes Jabar Jawa Barat.
7. Dinas Kesehatan Kota Cimahi. 2013. Profil Kesehatan Kota Cimahi tahun 2012, Dinkes Cimahi, Cimahi.
8. Dinas Kesehatan Kota Cimahi. 2015. Profil Kesehatan Kota Cimahi tahun 2014, Dinkes Cimahi, Cimahi.2015
9. Dinas Kesehatan Kota Cimahi. 2016. Profil Kesehatan Kota Cimahi Tahun 2015, Dinkes Cimahi.
10. World Health Organization. 2009. Dengue: guidelines for diagnosis, treatment, prevention, and control, WHO Geneva.
11. Hartoyo E. 2008. Spektrum klinis demam berdarah dengue pada anak, Sari Pediatri, 145-50.
12. Permatasari R, Aryati, Arifah B. 2015. Clinical pathology and majalah patologi klinik indonesia dan laboratorium medik, J Indonesia; 261-5.
13. Guzmán MG, Kourí G. 2004. Dengue diagnosis, advances, and challenges. Int J Infect Dis, 69-80.
14. Yatra IMS, Putra IWGAE, Pinatih GNI. 2014. Riwayat demam dengue dan keterlambatan diagnosis sebagai faktor risiko dengue shock syndrome di RSUD Wangaya Denpasar, Denpasar : Fakultas Kedokteran Udayana.
15. Dar W, Sofi P. 2015. A Rare Complication of Dengue Fever. J Gen Pract, 21-5.
16. Sutaryo. 2004. Penegakan diagnosis demem berdarah dengue, Yogyakarta : Medika Fakultas Kedokteran UGM.
17. Carrasco CD. 1927. Serological diagnosis. Tubercle, 171-2.
18. Focus Diagnostics. 2016. Dengue virus serology testing. Focusdx.com.
19. Dewi NLSP, Wirawati IAP. 2016. Peranan pemeriksaan serologi pada infeksi virus dengue. E-Jurnal Med Udayana,1-14.
20. Dewi, Ni Putu Eka Rosiana, A.A. Wiradewi Lestari WS tayasa. 2013. Karakteristik hasil uji antigen Non-struktural 1 (NS1) Pada pasien yang diduga demam berdarah dengue di laboratorium RSU Surya Husada periode Mei sampai Oktober: Fakultas Kedokteran Udayana.
21. Akualing, Jeine Stela. 2016. Diagnosis of dengue virus infection using IgA anti dengue rapid test. E-Cronicon, 688-696.
22. Andries AC, Duong V, Ong S. 2016. Evaluation of the performances of six commercial kits designed for dengue NS1 and anti-dengue IgM, IgG and IgA detection in urine and saliva clinical specimens. BMC Infect Dis, 1-9.
23. Board E. 2002. Advances In Parasitology. Berlin: J. Baker.
24. Wahid, Isra. 2002. Buku ajar parasitologi kedokteran, Makasar: Universitas Hasanudin, hal.81-9

25. World Health Organization. 2005. dengue, dengue haemorrhagic fever and dengue shock syndrome in the context of the integrated management of childhood illness department of child and adolescent health and development. Discuss Pap child Health Geneva.
26. Ahmed NH ussai., Broor S. 2014. Comparison of NS1 antigen detection ELISA, real time RT-PCR and virus isolation for rapid diagnosis of dengue infection in acute phase, J Vector Borne Dis; 194-9.
27. Da Costa VG, Marques-Silva AC, Moreli ML. 2012. a meta-analysis of the diagnostic accuracy of two commercial NS1 antigen ELISA tests for early dengue virus detection, PLOS One, 9.
28. Libraty DH, Young PR, Pickering D, et al., 2002. 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, 1165-8.
29. Kosasih H, Alisjahbana B, Widjaja S, et al., 2013. The diagnostic and prognostic value of dengue Non-Structural 1 antigen detection in a hyper-endemic region in Indonesia, PLOS One; 1-7.
30. Sharmin R, Tabassum S, Mamun KZ. 2012. Dengue-specific iga in different body fluids: a prospective alternative for dengue diagnosis in resource-poor settings. AMA; 124-133.
31. de la Cruz Hernández SI, González Mateos S, Flores Aguilar H, et al., 2012. Evaluation of a novel commercial rapid test for dengue diagnosis based on specific IgA detection. Diagn Microbiol Infect Dis; 150-155.
32. Naz A, Zahid D, Murky SN, Nadeem M, Sil BK, Shamsi TS. 2016. Evaluation of efficacy of various immunochromatographic rapid tests for dengue diagnosis. Pakistan Journal Medical Science; 166-171.
33. Nascimento, EJM., Huleatt JW, Cordeiro MT, et al. 2018. Development of Antibody biomarkers of long term and recent dengue virus infections. J Virol Methods.
34. Field, Andi., 2011. Discovering statistics using SPSS, London : SAGE Publication Ltd; p.148-155
35. Sopiyudin D., 2011. Statistik untuk kedokteran dan kesehatan, Edisi ke-5. Salemba Medika.
36. Sudigdo S,. 2011. Dasar Metodologi Penelitian Klinis, Edisi ke-4, Sagung Seto.
37. Puspitasari D., 2010. Profil antigen ns1 dengan hari sakit (los) pada infeksi virus dengue.
38. Setiawan M. 2012. Demam berdarah dengue (DBD) dan NS1 Antigen Untuk Deteksi Dini Infeksi Akut Virus Dengue. Jurnal Saintika Med.
39. Latief R. 2011. Nilai novel kombinasi nilai Antingen NS1 , Antibodi IgMdan IgG Anti Dengue Dalam Memprediksi Outcome Infeksi Virus Dengue di Makassar. Jurnal Pascasarja Universitas Hassanudin, 2011;1 :1-15.
40. World Health Organization, Marbawati D, Lescar J, et al. 2016. Evaluation of a novel commercial rapid test for dengue diagnosis based on specific IgAdetection. PLoS One, 2016;3:150-155.
41. Setiabudi D, Setiabudiawan B, Parwati I, and Garna H. 2013. The Difference of Platelet Activating Factor Plasma Level between Dengue Hemorrhagic Fever and Dengue Fever patients, 2013;45(4):251-256.
42. Jacob A, D. Pijoh V, and Wahongan. 2014. Ketahanan hidup dan pertumbuhan nyamuk Aedes sp pada berbagai jenis air perindukan. e-Biomedik; 1- 5.

AUTHOR BIOGRAPHY

Yudith Yunia Kusmala Faculty of Medicine, Universitas Jenderal Achmad Yani

Rini Sundari Faculty of Medicine, Universitas Jenderal Achmad Yani

Tezza Adriansyah Anwar Faculty of Medicine, Universitas Jenderal Achmad Yani

Jovy Yudha Tamba Medical Study Program, Faculty Medicine, Universitas Jenderal Achmad Yani