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Research Report

Relationship of Non Structural Antigen 1 (NS1) to Clinical Signs, Symptoms and Routine Blood Examination Dengue Suspected

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ABSTRACT

Early diagnosis of dengue infection is important due to very rapid disease patophysiology because late diagnostic can be fatal to the patient, remembered the journey of the disease is very rapid. Currently was a non-structural 1 dengue antigen (NS1) examination which can detect detect dengue viral infections earlier, even on the first day of fever. However, not all health care centers have adequate laboratory facilities for NS1 examination. Clinical symptoms and signs as well as a routine blood test are indicators that become basic of diagnosis in health care facilities with limited facilities. This study aims are to determine the relationship of NS1 examination to clinical sign and symptoms and NS1 examination as well as the result of routine blood tests in patients suspected dengue infection. This research was used observational analytic method with cross sectional approach. The research was conducted in clinic laboratory and inpatient clinic room of Vita Medika Kepung Kediri from November 2017 to February 2018.The number of research samples of 30 people was determined by the consecutive sampling technique. NS1 examination was done by using rapid immunochromatography test method with mono kits. Routine blood examination was done by using Micros 60. The reason for using chi square the test is because the two variables studied are variables with data in the form of a categorical scale Chi square test on relationship between clinical sign and symptoms examination of dengue with the results of NS1 examination obtained p= 0,310 (p > 0,005), while the results of chi square test on the relationship of routine blood examination results haemoglobine levels, amount of leucocyte, platelet count and of NS1 examination result obtained p value in a row p = 0,235 (p > 0,05), p = 0,013(p < 0,05), p = 0,028(p < 0,05) dan p = 0,132 (p > 0,05). There was a significant correlation between leucocyte count and platelet count to NS1 antigen exanimation result, but there was no correlation between clinical signs and symptoms of dengue patients, haemoglobine level and haematocryt value on NS1 antigen examination result.

Keywords: *Dengue Hemorragic Fever, Routine Blood Count , NS1 Antigent Dengue*

ABSTRAK

Penegakkan diagnosis infeksi dengue sejak dini sangat penting karena keterlambatan diagnosa dapat berakibat fatal bagi penderita , mengingat perjalanan penyakit ini yang sangat cepat. Saat ini telah dikembangkan suatu pemeriksaan terhadap antigen non struktural-1 dengue (NS1) yang dapat mendeteksi infeksi virus dengue dengan lebih awal bahkan pada hari pertama onset demam. Akan tetapi, tidak semua pusat layanan kesehatan, memiliki fasilitas laboratorium yang memadai untuk pemeriksaan NS1. Tanda dan gejala klinis serta pemeriksaan darah rutin merupakan indikator yang menjadi dasar diagnosis pada pusat layanan kesehatan dengan fasilitas yang terbatas. Penelitian ini bertujuan untuk mengetahui hubungan hasil pemeriksaan antigen NS1 terhadap gejala , tanda klinis dan hasil pemeriksaan darah rutin pada pasien terduga infeksi dengue. Penelitian ini menggunakan metode analitik observasional dengan pendekatan cross sectional. Penelitian dilakukan di Laboratorium Klinik dan Ruang Rawat Inap Klinik Rawat Inap Vita Medika Kepung Kediri pada bulan November tahun 2017 – Februari tahun 2018. Jumlah sampel penelitian 30 orang ditentukan dengan teknik consecutive sampling. Pemeriksaan antigen NS1 dilakukan menggunakan metode rapid immunochromatography test dengan kit Mono. Pemeriksaan darah rutin dilakukan menggunakan Micros 60. Uji chi square mengenai hubungan antara gejala dan

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tanda klinis dengue dengan hasil pemeriksaan NS1 diperoleh nilai p = 0,310 (p > 0,005) sedangkan hasil uji chi square mengenai hubungan hasil pemeriksaan darah rutin yaitu kadar hemoglobin , jumlah lekosit , jumlah trombosit dan nilai hematokrit terhadap hasil pemeriksaan NS1 didapatkan nilai p berturut – turut p = 0,235 (p > 0,05) , p = 0,013(p < 0,05) , p = 0,028(p < 0,05) dan p = 0,132 (p > 0,05). Terdapat hubungan yang bermakna antara jumlah lekosit dan jumlah trombosit terhadap hasil pemeriksaan antigen NS1, namun tidak terdapat hubungan antara tanda dan gejala klinis pasien dengue , kadar hemoglobin dan nilai hematokrit terhadap hasil pemeriksaan NS1.

Kata kunci: *Dengue, NS1,Pemeriksaan Darah rutin*

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INTRODUCTION

Dengue infection is the most common disease the tropical and subtropical district, especially Southeast Asia, central America, America and Carribian. The natural object of dengue is human, the agent is dengue virus that is included to family of Flaviridae and Flavivirus genus, contains of 4 serotipes for instance Den-1, Den-2, Den-3 and Den -41. The disease was transmitted to human through infected mosquito's bite, mainly *Aedes aegypti* mosquito and *Ae. albopictus* 2 that is nearly found in entire of Indonesia.[1]

Usually, Dengue patients were experienced the fever phase for 2-7 days, followed by critical phase for 2-3 days. The critical phase and occurs when patient has not in fever anymore, they in the phase patient will be at risk to get shock if do not get adequate treatment.[2] On dengue fever, after incubation intrinsic moment for 4-6 days, appears non-specific clinical symptom, constitutional symptom, headache, backache and malaise. Dengue bleed fever is indicated with two or more clinical manifestation as follows: headache, retro orbital ache, rash, antralgya and mialgya, bleeding manifestation (positive tourniquet test, petekie), leukopenia and positive dengue serology examination. Onset Dengue is usually marked by high fever, headache and flushing.[3]

In general, the diagnosis of dengue is difficult to enforce in the first few days of illness because the symptoms that appear are not specific and difficult to distinguish from other infectious diseases.[4] Diagnosis of dengue infection only based on clinical syndromes which cannot be fully trusted, so the diagnosis needs to be confirmed using laboratory tests. Laboratory tests that can be done to diagnose dengue infection include: virus isolation, detection of viral nucleic acids, detection of viral antigens, tests based on immunological responses (anti-dengue IgM and IgG), and hematological parameter analysis.[5]

Hematologic parameters that are routinely examined to screen suspected patients with dengue fever are through examination of hemoglobin levels, leucocyte counts, hematocrit values, platelet counts, and peripheral blood smears to see the presence of relative lymphocytosis and blue plasma lymphocyte.[6]

Nowadays NS1 antigen examination has been developed to detect the presence of dengue virus infection in the acute phase, where in various studies it has been shown that NS1 is superior in sensitivity than viral culture and *polymerase chain reaction* (PCR) examination as well as antidengue IgM and IgG antibodies. The bag-specific NS1 antigen 100% is as high as the gold standard for viral culture or PCR.[7]

Dengue virus has two types of proteins, namely structural envelope (E) proteins, matrix (M) and capsid (C)) and nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) .[8,23] Protein E, Pr M protein and NS1 protein has antigenic properties.[9] Nonstructural protein NS1 in the dengue virus is a glycoprotein measuring 46-50 kilodalton expressed on infected host cells both membrane associated (mNS1) and secreted (sNS1) and not part of the virion structure component.[10] NS1 is produced by all flaviviruses and plays an important role in the process of

replication and survival of the virus.[11] NS1 acts as an important immunogen in dengue infection and plays a role in protecting against diseases, especially in secondary infections where anti-CSF antibodies are found in the patient's serum. pathogenesis of dengue infection.[12]

In addition to the viral bond with antibodies, NS1 antigens also play a role in plasma leakage and bleeding in dengue infections. NS1 antigen will bind to specific antibodies resulting in vascular endothelial cell apoptosis and activation of the complement system which contributes to plasma leakage and platelet lysis.[13]

However, NS1 examination is rarely done in health laboratories, especially in rural areas, this is one of them because the examination price is quite expensive. Routine blood tests which include hemoglobin examination, leukocytes, platelets and hematocrit are determinants in diagnosing other than clinical symptoms.[14]

Based on the background above, in this study the researchers intend to find out whether or not there is a relationship between NS1 examination results and clinical signs and symptoms as well as routine blood examination results which include hemoglobin level, leukocyte count, platelet count and hematocrit value in patients suspected of dengue infection.

MATERIALS AND METHODS

This type of research is an observational analytic study with a cross sectional approach. This study aims to determine the relationship between NS1 antigen examination results on clinical symptoms, platelet count and hematocrit values in patients suspected of dengue infection clinic Vita Medika Kepung, Kediri Regency. The research was conducted in November 2017 - February 2018 in the Clinical Laboratory section and inpatient Vita Medika Kepung clinic in Kediri Regency.

The sample used in this study were patients with suspected dengue infection at the Vita Medika Kepung clinic in Kediri Regency in November 2017 - February 2018 with inclusion criteria as stated bellow male and female sex, age 0-10, 11-20, 21-30, >30 year,[4] (with total of 30 subjects for all classifications), illness duration at hospital admission <5 days from the onset of fever, no other illness indications, not consuming any medicine which suppress spinal cord, complete medical record, no blood deviation, willing to be research subject. The sampling technique in this study was consecutive sampling. At consecutive sampling, all subjects who arrived and met the selection criteria were included in the study until the required number of subjects were met. This consecutive sampling is the best type of non-probability sampling and is the easiest method. Most clinical studies (including clinical trials) use this technique to select subjects. The number of samples was determined a formula.

Exclusion criteria in this study were patients with suspected dengue with a long illness since the onset of fever for more than 5 days, patients who were taking medications that suppressed bone marrow, patients who had a history of blood disorders, patients with other coincidental diseases, such as typhoid fever, patients with indications of other infectious diseases, such as respiratory infections, urinary tract infections and gastrointestinal infections, incomplete medical records, patients with symptoms and signs of shock, and unwilling to become respondents in the inform consent.

The independent variable (independent variable) in this study is the results of nonstructural antigen 1 (NS1). The dependent variable in this study is the clinical signs and symptoms of dengue infection, hemoglobin level, leukocyte count, platelet count and hematocrit value. Clinical symptoms referred to in this study are fever, which is accompanied by at least 2 of the following symptoms: headache, retroorbital pain, myalgia, arthralgia, rash, and bleeding manifestations such as petechiae, positive tourniquet test, and spontaneous bleeding.

Data processing including the examination of the completeness and clarity of the data, assigning code to each variable data, entering data in the SPSS program (Statistical Program for Social Science), and checking back to ensure that the data has been cleared of errors. Data analysis consisted of univariate and bivariate analysis. The

statistical test used in this study is the chi square test. The reason for using the test is because the two variables studied are variables with data in the form of a categorical scale. If the chi square test does not meet the requirements (the expected count value is less than 5> 20%), then use the Fisher exact test for 2x2 tables, Kolmogorov Smirnov test for 2x2 tables.

RESULTS AND DISCUSSION

In this part, we provide the research result, it consists of 12 tables.

Table 1 was showed that in this study it was found that according to gender the number of male respondents were more than female respondents with a ratio of 1.14: 1. These results are in line with the results of research conducted by Libraty[6,8,24] who get more male sufferers than women with ratio of 2.2: 1, as well as in the research conducted by Mayer *et al*, [15] the number of male respondents was more than women with a ratio of 3: 2 and research by Juranah[21] in 2011. Production of anti inflammation sitocyn in female was more abundant, therefore, female who get dengue infection give unclear clinical complaint and are rarely to be hospitalized or clinic.[8]

In women the production of anti-inflammatory cytokines is greater, so that women infected with dengue provide clinical complaints that are less clear and rarely treated in hospitals or clinics.[2] This is also confirmed by Soedarmono et al[17] who stated that the XX chromosome in women has a role in managing immunoglobulin production quantitatively. But Halstead et al[18] research shows that there is no difference between the response of infection in women and men.

Based on the age in this study found the youngest respondents in this study were 3 years and the oldest 38 years, the highest percentage of 15 (50%) respondents were children aged <10 years, followed by respondents with the age group 11-20 years as many as 8 (26.7 %). The results of this study were supported by a statement from the Caribbean Epidemiology Center in 2000, which stated that the most epidemiology of dengue patients was in children and young adults. Age is one of the factors that influence sensitivity to dengue virus infection.[18] Study was conducted in Kuba which showed that age had an important role for the emergence of clinical symptoms in the form of plasma leakage.[19]

Table 2 is showed that from 30 respondents as many as 25 (83.3%) experienced clinical symptoms of dengue, namely fever (in this case selected respondents were those who had fever 1 - 4 days), headaches, joint pain, nausea without signs of bleeding and 5 (16, 7%) of the respondents accompanied by a sign of bleeding which is positive for Rumple Leed (RL) examination. These results indicate that often the initial clinical symptoms of dengue infection are not typical, as evidenced by the variation in clinical symptoms experienced by respondents.

From 25 respondents who do not show bleeding sign, 14 of them (56%) has positive NS1 antigen and 11 of them (44%) has negative NS1 antigen. While in 5 respondents who show bleeding signs, there are 3 respondents (60%) has positive NS1 antigen and 2 respondents (40%) has negative NS1 antigen. Chi square test result between respondents clinical symptom when

Table 1. Characteristics of Research Subject

Characteristics	N	%
Gender		
Male	16	53,3
Female	14	46,7
Range of age (years old)		
0 - 10	15	50
11 - 20	8	26,7
21 – 30	3	10
>30	4	13,3
Total	**30**	**100**

Adv: n=frequency

Table 2. Distribution of Clinical Signs and Symptoms of Patients Suspected of Dengue Infection during Admission

Clinical symptoms and Signs of dengue (fever, arthritis, headache, nausea)	n	%
Without bleeding signs	25	83,3
With bleeding signs	5	13,3

Adv: n=frequency

admission toward NS1 antigen checking result obtain p value = 0.310 (p > 0.05) means that there is no correlation between signs and clinical symptoms and NS1 antigen checking result. This result is in line with research Muhamad[12] in 2007 who claims that there is no correlation between symptoms and clinical signs with NS1 antigen checking result with p value = 0.115 (p > 0.05).

Table 3 was described that at the time of admission from 30 respondents there were 7 (23.3%) respondents had Hb <normal, 23 (76.7%) respondents had Hb levels within the normal range, and no (0%) respondents had Hb levels> normal. In this study the determination of normal values is distinguished by the sex of the respondents.

Chi square result between Hb and NS1 antigen checking result obtains p value = 0.235 (p > 0.05) which has meaning that there is no correlation between Hb and NS1 antigen checking result. This result is in line with research[10] conducted in 2016 which found that there is no correlation between Hb of patients suspected with dengue infection and NS1 antigen checking result with p value = 0.483 (p > 0.05).

Table 4 was showed that in this study, at the time of admission, 22 (73.3%) respondents had leukocyte counts <4,000 cells / mm3 (<Normal), and as many as 8 (26.7%) respondents had leukocytes between 4,000 - 10,000 cells / mm3 (Within Normal Limit) and no respondent experienced an increase in leukocyte count. This shows a tendency to decrease the number of leukocytes in the early phase of dengue infection.

Chi square result between leucosite amount and NS1 antigen checking obtains p value = 0.013 (p < 0.05) which shows that there is significant relationship between leucosite amount and NS1 checking result which is decreasing of leucosite amount and NS1 positive checking result. Similar result was claimed by a research Irawan[10] in 2016 which states that there was a correlation between leucosite and NS1 antigen checking result with p value = 0.000 (p < 0.05)

Table 5 was illustrated that at the time of admission as many as 13 (43.3%) respondents had platelet counts <100,000 cells / mm3d and 17 (56.7%) respondents still had a platelet count> 100,000 cells / mm^3 and of the 17 respondents who had platelet counts> 100,000 cells / mm^3 there were 4 (13.3%) having normal platelet counts. These results were indicated that in the initial phase of infection some respondents experienced thrombocytopenia and some did not / had not experienced tombocytopenia. Thrombocytopenia usually occurs after the onset of heat on the 3rd - 7th day. Respondents who

Table 3. Distribution of Hemoglobin Levels in Patients Suspected of Dengue Infection during Admission

Hemoglobin Levels (g/dL)	N	%
Male		
< 13,5	7	23,3
13,5 – 18,0	9	30,0
> 18,0	0	0
Female		
< 11,5	0	0
11,5 – 16,0	14	46,7
> 16,0	0	0
Total	**30**	**100**

Advt: n=frequency

Table 4. Distribution of Patient Leukocytes Suspected of Dengue Infection during Admission

Leukocyte count (sel/mm^3)	N	%
< 4.000	22	73,3
4.000 – 10.000	8	26,7
>10.000	0	0
Total	**30**	**100**

Adv: n=frequency

Table 5. Distribution of Patient's Platelet Number Suspected of Dengue Infection during Admission

Platelet count (sel/mm^3)	N	%
<100.000	13	43,3
>100.000	17	56,7
Total	**30**	**100**

Adv: n=frequency

have not experienced thrombocytopenia may not have entered the platelet decline phase.

Chi square test result obtains p value = 0.028 (p > 0.05) which shows there is significant result between trombocite amount and NS1 antigen checking result, where the trombocite decreasing is in line with NS1 positive antigen checking result, however, there are some subjects who did not experienced trombocite decreasing.

This result is in line with a research Muhamad[12] in 2017 which claimed that there was a significant relationship between trombocite amount and NS1 antigen checking result with p value = 0.031 (p < 0.05).

In Table 6 it can be seen that at 3 (10%) the respondents had hematocrit values below normal, 10 (33.3%) respondents had normal hematocrit values and 17 (56.6%) respondents had hematocrit values above normal. This shows that most respondents experienced an increase in hematocrit values during admission. But if it is associated with the criteria for dengue diagnosis applied by WHO that is an increase in hematocrit value > 20%, then there are only 5 (16.7%) respondents who meet these criteria.

This result is in line with the research conducted Irawan [10] in 2016 which claimed that there is no significant result between hematocrite and NS1 antigen checking result with p value = 0.810 (p > 0.05).

Table 7 was described from 30 respondents found 17 (56.7%) had positive NS1 antigen examination results and 13 (43.3%) had NS1 negative antigen examination results. The existence of negative results in this study could be due to misinformation regarding the length of fever experienced by respondents (fever > 4 days) so that the NS1 antigen was undetectable or it could also be because the respondent was really not infected with dengue, therefore further investigation is needed. Namely serological examination of dengue Ig M and IgG which usually begins to be detected on days 5 - 10 of fever (in the confaleen phase).[15]

At present, an examination of dengue antigen has been developed, namely non-structural 1 dengue antigen (NS1 antigen) which can detect dengue virus infection earlier even on the first day of onset of fever.[16,22]

Table 6. Distribution of Hematocrit Value in Patients Suspected of Dengue Infection during Admission

Hematocrit Value (%)	N	%
Adult Male		
<40	1	3,3
40-48	2	6,7
>48	2	6,7
Adult Female		
<37	1	3,3
37-43	1	3,3
>43	3	10,0
Kids		
<= 15 years old		
< 33	1	3,3
33 – 38	7	23,4
>38	12	40,0
Total	**30**	**100**

Adv: n=frequency

Table 7. Distribution of NS1 Antigen Examination Results in Patients Suspected of Dengue Infection during Admission

NS1 Antigen	n	(%)
Positive	17	56,7
Negative	13	43,3
Total	**30**	**100**

Adv: n=frequency

NS1 is a non-structural glycoprotein with a molecular weight of 46-50 kD and is a highly conserved glycoprotein. Initially NS1 was described as a Soluble Complement Fixing (SCF) antigen in the culture of infected cells. NS1 is needed for the survival of the virus but its biological activity is unknown. Existing evidence shows that NS1 is involved in viral replication. NS1 itself is produced in two forms: membrane associated and secreted form. During cell infection, NS1 is found to be associated with intracellular organelles or transferred via secretion pathways to the cell surface (cytoplasmic membrane). NS1 is not part of the structure of the virus, but it is excreted on the surface of infected cells and has group-specific determinants and types. The role

of NS1 in immunopathogenesis has also been submitted based on the findings of anti-SCF antibodies in serum patients with secondary infection but not in primary infection.[17]

NS1 dengue is secreted into the blood system in individuals infected with the dengue virus. NS1 circulates at high concentrations in the serum of patients with primary and secondary infections during the clinical clinic phase (Clinical Phase of Illness) and the first days of the convalescence phase (recovery).[18] From the results of the study it was also shown that NS1 detection can provide a specific diagnosis of dengue infection.[10]

Datta and friends in India in 2010 were, compared NS1 in the acute phase was NS1 positive 71.42% in the acute phase, while in the NS1 positive convalescence phase only 6.38%. High sensitivity in the initial phase of fever because NS1 protein circulates in high concentrations in the patient's blood during the initial acute phase, both in primary infection and in secondary infection. The high level of NS1 until day 5 of fever is related to the time of virenia because it is a period of viral replication and the absence of antibodies against the virus. Levels of virenia and NS1 levels also depend on intrinsic characteristics and strains of the virus that infects and immunity status of the patient itself.[11]

Another study was conducted by Kumarasamy et al., It was obtained the results that the sensitivity of commercial reagents for NS1 dengue antigen for acute dengue infection was 93.4% and specificity was 100%. Positive and negative forecast values are 100% and 97.3%, respectively. Lastere et al studied 181 patients with DHF in France polynesia found NS1 sensitivity of 76.5% and specificity of 96.2%.[12]

Table 8 was showed that in this study obtained from 25 respondents who showed no signs of bleeding found 14 (56%) respondents had positive NS1 antigen examination results and 11 (44%) respondents had negative NS1 antigen examination results. While the 5 respondents who showed signs of bleeding found 3 (60%) had a positive NS1 examination results and 2 (40%) had negative NS1 antigen examination results. Chi square test results between respondent

Table 8. Relationship between Clinical Signs and Symptoms and Results of NS1 Antigen Examination

Clinical symptoms and signs Dengue (Fever, Headache, Arthritis, Nausea)	NS1 Antigen		Total	Value P	PR (ik95%)
	Negative	Positive			
Bleeding signs n (%)	11	14	25		
	(44,0)	(56,0)	(100)		
With bleeding signs n (%)	2	3	5	0,310	1,071
	(40,0)	(60,0)	(100)		
	13	17	30		
Total	**(43,3%)**	**(56,7%)**	**(100%)**		

Adv: n=frequency

clinical signs and symptoms during admission of NS1 antigen examination results were obtained p value = 0.310 (p> 0.05) which means there is no relationship between clinical signs and symptoms with NS1 antigen examination results. The results of this study are in line with the research[12] in 2007 which states there is no relationship between symptoms and clinical signs with NS1 antigen examination results with p = 0.115 (p> 0.05).

Signs and clinical symptoms typical of dengue infection are signs of bleeding, the most are skin bleeding such as a torniquet test (positive RL test, weir test), but not all of the signs of bleeding occur in dengue patients.[15] Torniquet test is positive if there are more than 10 petechiae in a diameter of 2.5 cm at the bottom of the front (volar) including the elbow fold (cubital fossa). A positive tourniquet test shows that capillary fragility is increased, but this condition can also be found in diseases caused by other viral infections such as measles, chikung fever, and abdominal typhus bacterial infection.[7] The presence of a variety of early clinical signs and symptoms that are not typical often results in delays in diagnosis. The course of this disease can be very fast in a few days, even in a matter of hours sufferers can enter the critical phase. To avoid delays in diagnosis, physical examination and anamnese alone are not enough, it is necessary to do other examinations, namely laboratory tests as a supporter as well as enforcement of the diagnosis.

Table 9 was showed that in this study the results obtained when the admission of 30 respondents there were 7 (23.3%) respondents had a normal Hb level and 23 (76.7%) respondents had a normal Hb level.

Of the 7 respondents who had <normal 3 hemoglobin levels (42.9%) had a positive NS1 examination result and 4 (57.1%) had a negative NS1 antigen examination result. Chi square test results between hemoglobin levels and NS1 antigen examination results obtained values p = 0.235 (p> 0.05) which means there is no relationship between hemoglobin levels and NS1 antigen examination results. These results are in line with research conducted by Irawan Anasta Putra, et al in 2016 which stated that there was no relationship between hemoglobin levels in patients with suspected dengue infection and NS1 antigen examination results with p = 0.483 (p> 0.05).

Hemoglobin is a molecule consisting of heme (iron) and globin polypeptide chains (alpha, beta, gama and delta), are in the erythrocytes and are responsible for transporting oxygen.[8] Blood quality is determined by hemoglobin levels. The structure of Hb is expressed by mentioning the number and type of globin chains that exist. There are 141 amino acid molecules in the alpha chain and 146 amino acid molecules in the beta chain, gama and delta. Hb levels in the first days of the dengue infection are usually normal/slightly decreased, but then the levels will increase following the increase in hemoconcentration.[15]

Table 10 is showed that in this study the results obtained at the time of admission as many as 22 (73.3%) respondents had leukocyte counts <4,000 cells/mm^3 (<Normal), and as many as 8 (26.7%) respondents had a leukocyte count of 4,000 - 10,000 cells/mm^3 (Within Normal Limit) and no respondent experienced an increase in leukocyte count. Chi square test results regarding the relationship of leukocyte count and NS1 antigen examination results obtained p value = 0.013 (p < 0.05) which indicates that there is a significant relationship between leukocyte count and NS1 antigen examination results in a decrease in the number of leukocytes proportionally/in line with the results of the examination NS1 antigen is positive.

Similar results were revealed by Hottz, et al,[19] which in his research found the results of leukocyte counts with NS1 antigen examination results with p = 0.000 (p <0.05). Lecocytes are white blood cells that function as the body's defense against bacteria or viruses in the body. Normal levels of leukocytes range from 4,000 - 10,000 cells/ mm^3.Lecocytes also have an important role in the body's immunological function, if there is an increase in leukocytes can be used as a sign that infection occurs in the body. [20]

At the beginning of dengue infection (when the fever starts) the leukocyte count is usually normal or decreases with neutrophil cell dominated. The occurrence of lecopenia is mainly caused by mature PMN (Polymorphonuclear) leukocytes

Tabel 9. Relation of Hemoglobin Levels to NS1 Antigen Examination Results

Hb levels	NS1Antigen		Total	Value p	PR (ik95%)
	Negative	Positive			
< Normal n (%)	4	3	7		
	(57,1)	(42,9)	(100)		
Normal n (%)	9	14	23	0,235	1,420
	(39,1)	(60,9)	(100)		
	4	3	7		
	13	17	30		
Total	**(43,3%)**	**(56,7%)**	**(100%)**		

Adv:n=frequency

Table 10. The Relationship of Leukocyte Count with NS1 Antigen Examination Results

Hb value	NS1Antigen		Total	Value P	PR (ik95%)
	Negative	Positive			
< Normal n (%)	6	16	22		
	(27,3)	(72,7)	(100)		
Normal n(%)	7	1	8	0,013	5,816
	(87,5)	(12,5)	(100)		
	13	17	30		
Total	**(43,3%)**	**(56,7%)**	**(100%)**		

Adv: n= frequency

production, while in the last phase pain is found to increase lymphoblastoid cells.[11] Lekopenia reaches its peak just before the fever drops and is normal again in 2-3 days after defervescence.[12] Increased lymphoblastoid cells at the end of dengue disease originate from the transformation of T cells in leukocytes. T cells play a role in cellular immuno responses, recognize and destroy virus-infected cells and activate macrophages in phagocytosis due to immunological stimulation of dengue infection. [20]

Table 11 was showed that in this study results were obtained, when the admission of 13 (43.3%) respondents had platelet counts <100,000 cells/ mm^3 and 17 (56.7%) respondents still had a platelet count> 100,000 cells / mm^3

And of the 17 respondents who had platelet counts> 100,000 cells / mm^3 there were 4 (13.3%) having normal platelet counts. Of the 13 responses that had platelet counts <100,000 cells/mm^3, 9 (69.2%) responses had a negative NS1 antigen examination result and 4 (30.8%) had positive NS1 antigen examination results.

While in respondents with platelet counts> 100,000 cells / mm3 there were 4 (23.5%) had negative NS1 antigen examination results and 11 (76.5%) had positive NS1 antigen examination results. The chi square test results obtained by shine p = 0.028 (p <0.05) which showed a significant relationship between platelet counts and NS1 antigen examination results, where the decrease in platelets was in line with the positive NS1 antigen examination results, but there were some who did not experience a decrease in platelets have positive NS1 antigen examination results. This result is similar to the results of research conducted by Muhamad[12] which states there is a significant relationship between platelet counts and NS1 antigen examination results with p = 0.031 (p <0.05). Thrombocytopenia has an important role in the pathogenesis of dengue infection. Thrombocytopenia in dengue infection occurs through the mechanism of bone marrow suppression, platelet destruction and shortening of platelet life. In this study, the lowest platelet count occurred on day 4 since the onset of fever and decreased platelet count (00150000 cells/mm^3) generally occurred on the 2-3th day since the onset of fever. Decreased platelet count to .000100,000 cells/mm^3 or less than 1-2 platelets / large field of view (LPB) with the average inspection carried out at 10 lpb. In general thrombocytopenia occurs before there is an increase in hematocrit and occurs before the temperature drops. Platelet count Ý100,000/ mm^3 is usually found between days 3 - 7.[14] Platelet count can be used as a tool to diagnose dengue because it shows high sensitivity from day 4 of fever at 67.7%, even on day 5 to 7th shows 100%. Very high specificity in the use of thrombocytopenia as a parameter is caused by infrequent infectious diseases accompanied by a decrease in platelet count below 150,000 cells/mm^3. Even if the criteria for thrombocytes below 100,000 cells/mm^3 are used, the specificity is almost 100% from the first day, but reduces the sensitivity between 10-20%. 15 Thus the daily platelet examination will greatly help the diagnosis of dengue because it increases its sensitivity and specificity. [13]

Table 12 was showed that the results of the study were obtained during admission as many as 3 (10%) respondents had hematocrit values below normal, 10 (33.3%) respondents had normal hematocrit values and 17 (56.6%) respondents had hematocrit values above normal. This shows that most respondents experienced

Table 11. Relationship of Platelet Amounts with NS1 Antigen Examination Results

Platelet Amount (sel/mm^3)	NS1 Antigen		Total	Value P	PR (ik95%)
	Negative	Positive			
<100.000 n (%)	9	4	13		
	(69,2)	(30,8)	(100)		
>100.000 n (%)	4	13	17		1,400
	(23,5) 13	(76,5) 17	(100) 30		
Total	**(43,3%)**	**(56,7%)**	**(100%)**		

Adv: n=frequency

an increase in hematocrit values at the beginning of dengue infection. But if it is associated with dengue diagnosis hematocrit criteria applied by WHO that is an increase in hematocrit value > 20%, then there are only 5 (16.7%) respondents who meet these criteria. From the results of the chi square test to determine the relationship between hematocrit values with NS1 examination results, hematocrit values in this study were divided into two groups, namely the group with hematocrit value < 39% and the group with hematocrit value> 39%. From the results of the grouping on the chi square test obtained 11 (36.7%) respondents had a hematocrit value <39% where 5 (45.5%) respondents had negative NS1 antigen examination results and 6 (54.5%) respondents had antigen examination results NS1 is positive. And in groups with hematocrit value> 39%, 19 (63.3%) respondents divided into 8 (42.1%) respondents had NS1 negative antigen examination results and 11 (57.9%) respondents had positive NS1 antigen examination results. The results of the chi square test obtained p value = 0.132 (p> 0.05) which means there is no relationship between the hematocrit value of respondents during the admission with NS1 antigen examination results. This result is in line with the results of research Irawan[10] which states that there is no significant relationship between the matrix value and the NS1 antigen examination results with p value = 0.810 (p> 0.05).

Table 12. Relationship of Hematocrit Value with NS1 Antigen Examination Results

Hematocrit value (%)	NS1 Antigen		Total	Value P	PR (ik95%)
	Negative	Positive			
< 39 n (%)	5 (45,5)	6 (54,5)	11		
>=39 n (%)	8 (42,1)	11 (57,9)	19	0,132	1,062
	13	17	30		
Total	**(43,3%)**	**(56,7%)**	**(100%)**		

Adv: n=frequency

In general, a decrease in platelets precedes an increase in hematocrit. In dengue infection, hematocrit values usually begin to increase on day 3 of the course of the disease and increase according to the process of dengue disease.[18] Increased hematocrit value is a manifestation of hemoconcentration that occurs due to plasma leakage into the extra vascular space with serous fluid effusion through damaged capillaries. As a result of this leakage, plasma volume is reduced, resulting in hypovolemic shock and circulatory failure. In severe cases accompanied by bleeding, generally the hematocrit value does not increase even decreases.[14] Regular examination of hematocrit is needed in the treatment of dengue infection so as to prevent the possibility of hypovolemic shock that causes blood circulation failure.

CONCLUSION

In conclusion, there is no significant correlation between signs, clinical symptoms, and haemoglobyn level in dengue suspected patient, moreover, there is significant correlation with the amount of leucosyt, trombocyt, hematocryt toward dengue infection. Because of that, we will be able to diagnose dengue patient, as a result, we will be able to clinical symptoms in more detail way.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest for this research.

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