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Research Article

OVERVIEW OF NUCLEAR FACTOR-κB (NF-κB) AND NON-STRUCTURAL PROTEIN 1 (NS1) IN PATIENTS WITH DENGUE FEVER IN PREMIER HOSPITAL, SURABAYA

Ni Nyoman Budiutari[1a], Yoes Prijatna Dachlan[2], dan Jusak Nugraha[3]
[1]Department of Immunology Postgraduate School, Universitas Airlangga, Surabaya, East Java, Indonesia
[2]Departement of Parasitology, Faculty of Medicine, Universitas Airlangga Surabaya, East Java, Indonesia
[3]Departement of Patology Clinic, Faculty of Medicine, Dr. Soetomo Hospital, Surabaya, East Java, Indonesia
[a]Corresponding author: budiutari2212@gmail.com

ABSTRACT

Dengue fever (DF) is an acute viral fever caused by RNA virus that is transmitted by Aedes aegypti and Aedes albopictus mosquitoes. DF is also called viral arthropod-borne disease and is accompanied by headaches, joint and muscle pain. The main target of dengue infection is macrophages or monocytes and dendritic cells (DC). Infected DC is caused the viral replication and the endocytosis into endosomal, easier, thus inducing the activation of NF-κB transcription factor to produce proinflammatory cytokines such as Tumor Necrosis Factor-α (TNF-α), Interleukin-1 (IL-1), IL-6, IL-12 and chemokine. NF-kB is one of the transcription factors involved in the regulation of the expression of various cytokines, chemokines and anti/pro-apoptotic proteins during infection and act as indicator of disease severity. Infected DC cells are secreted NS1 protein which is the co-factor needed for viral replication and can be detected in the first eight days. The level will be higher in the initial phase of fever. The purpose of this study was to analyze the description of NF-kB and NS1 levels in the serum of patients with dengue fever through observational analytic studies through a cross-sectional approach. This study was done on 40 patients with dengue fever and 10 healthies people as negative controls. NS1 was analyzed in serum of Panbio rapid test and NF-kB level were measured by sandwich ELISA. The results are showed positive and negative NS1 results in dengue fever patients. The average NF-kB serum level in dengue fever patients was found to be higher than the control. NF-κB level in negative NS1 was higher than the NS1 positive group. It is showed that NS1 is detected both in the acute phase. The detection of NF-κB is showed the involvement of transcription factors in the development of dengue virus infection and has a protective role for host cells.

Keywords: *Dengue Fever, Nuclear Factor-κB, NS1 Protein, Viral Infection, Tropical Disease.*

ABSTRAK

Demam Dengue (DD) merupakan penyakit demam virus akut yang disebabkan oleh virus RNA, ditularkan oleh nyamuk Aedes aegypti dan Aedes albopictu. DD disebut juga sebagai penyakit arthropod-borne viral dan disertai sakit kepala, nyeri otot dan sendi. Target utama infeksi dengue adalah makrofag/monosit dan sel dendritik (DC). DC yang terinfeksi akan mempermudah replikasi virus dan endositosis ke dalam endosomal, dimana hal tersebut dapat menginduksi aktivasi faktor transkripsi NF-κB untuk menghasilkan sitokin proinflamasi seperti Tumor Necrosis Factor-α (TNF-α), Interleukin-1 (IL-1), IL-6, IL-12 dan kemokin. NF-kB merupakan salah satu faktor transkripsi yang terlibat dalam regulasi ekspresi berbagai sitokin, kemokin, protein anti/pro-apoptosis selama infeksi dan menjadi tanda tingkat keparahan penyakit. Sel DC yang terinfeksi akan mengsekresikan protein NS1 yang merupakan co-factor yang diperlukan untuk replikasi virus. NS1 terdeteksi baik dalam delapan hari pertama serta kadarnya akan lebih tinggi pada fase awal demam. Tujuan penelitian ini adalah menganalisis gambaran kadar NF-kB dan NS1 pada serum pasien demam dengue melalui studi observasional analitik melalui pendekatan cross sectional. Pada penelitian ini menggunakan 40 sampel penderita demam dengue dan 10 sampel orang sehat sebagai control negatif. NS1 dianalisa pada serum menggunakan Panbio rapid test dan kadar NF-kB menggunakan sandwich ELISA. Hasil menunjukkan pada pasien demam dengue ditemukan hasil NS1 positif maupun negatif. Rerata kadar NF-kB serum pada pasien demam dengue ditemukan lebih tinggi daripada kontrol dan kadar NF-κB pada NS1 negatif lebih tinggi dibandingkan

kelompok NS1 positif. Hal ini menunjukkan bahwa NS1 terdeteksi baik pada fase akut dan terdeteksinya NF-κB menunjukkan adanya keterlibatan faktor transkripsi pada perkembangan infeksi virus dengue dan mempunyai peran proteksi untuk sel host.

Kata kunci: *Demam Dengue, Nuclear Factor- κB, Protein NS1, Infeksi Virus, Penyakit Tropis.*

INTRODUCTION

Dengue is an endemic disease caused by RNA viruses which transmitted by Aedes aegypti and Aedes albopictus mosquitoes.[1] This disease is endemic throughout tropical and subtropical regions which are affected by rainfall, temperature and unplanned urbanization.[2] World Health Organization (WHO) is recorded from 1968 to 2009 Indonesia as the country with the highest DHF cases in Southeast Asia. In Indonesia, it was first discovered in the city of Surabaya in 1968 with 58 people infected and 24 people dead. In 2015 there were 126,675 sufferers in 34 provinces in Indonesia.[3] In Surabaya in 2015 there was an increase in the number of cases by 46%.

Dengue Fever (DD) is an endemic disease which is also called viral arthropod-borne disease. The first infection to the host's body raises various pathological levels, ranging from asymptomatic mild symptoms (dengue fever), Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS).[4] Dengue is classified as a pediatric disease in Southeast Asia, but at this time dengue patients are recorded not only in children but also in adults.[5]

Dengue virus (DENV) is a genus of Flavivirus in the family Flaviviridae which has four different antigenic serotypes namely DENV-1, DENV-2, DENV-3 and DENV-4.[6] The DENV genome consists of positive single-stranded RNA with a length of about 11 kb which encodes ten proteins, ie no protein structural capsules (C), envelope (E), pre-membrane (preM) and seven non-structural proteins (NS1, NS2A, NS3, NS4, NS4A, NS4B, NS5).[2]

Non-structural 1 (NS1) protein is an important glycoprotein and a co-factor needed for viral replication, although its role in replication is largely unknown. NS1 is detected during the acute phase and will slowly decrease to an undetectable level on days 5-6. The presence of this protein is associated with the severity of the disease and the progression towards DHF.[6] The main target in dengue infection is towards macrophages/monocytes and dendritic cells.[7] Dendritic cells were infected with dengue virus will facilitate viral replication and endocytosis to endosomal, which can induce activation of the NF-κB transcription factor.[8]

NF-κB is a transcription factor that is involved in the regulation of gene expression to encode cytokines, chemokines, proproteins and antiapoptosis. Activation of NF-κB during viral infection is explained as a host protective response to pathogens. NF-κB is a weapon used by hosts to control viruses, but viruses can use them differently to block apoptosis and increase viral replication. Activation of the NF-κB pathway produces various cytokines such as Tumor Necrosis Factor-a (TNF-α), Interleukin-1 (IL-1), IL-6, IL-12. NF-κB is one of the most widely exploited pathways for gene regulation by DENV, but how the role of the NF-κB pathway in dengue pathogenesis is not completely clear.[9]

This study is based on the picture of NF-κB levels and NS1 protein in serum of dengue fever patients and controls, where an increase in NF-κB levels is indicated as a sign that a more severe prognosis is Dengue Shock Syndrome (DSS).

MATERIAL AND METHOD

Study Population

This study was done in the Institute of Tropical Diseases, Universitas Airlangga on August 2018. The study was included 40 patients with dengue fever and 10 healthy people as control who were selected from Surabaya Premier Hospital. Patients who were selected were in accordance with the inclusion criteria in patients with clinical symptoms such as that had onzet fever day 1–4 and without age limitation. Patients who had only clinical symptoms of dengue, but dengue infection had not found in diagnosis laboratory were excluded because of the difficulty of evaluating the extent of the disease.

The control group was included 10 apparently healthy subject with comparable age characteristics, no positive history of dengue infection disease, no fever for 1 month prior to the study.

The clinical disease severity was classified according to the 2011 World Health Organization (WHO) dengue diagnostic criteria.[10] Patients with sharp temperature and is frequently associated with a flushed face and headache, the body temperature is between 39-40°C and lasting 5-7 days in the majority of cases and the other common symptoms including anorexia were classified as DF. Patients with increase of hematocrit greater than 20% compare with the baseline hematocrit or clinical/ultrasound scan evidence of plasma leakage were classified as having DHF. Shock was defined as having cold clammy skin, along with a narrowing of pulse pressure of 20 mmHg. According to the WHO 2011 disease classification, 40 patients were classified as DF.

Ethics Statement

Ethics approval was obtained by Ethical Review Committee at Faculty of Dental Medicine, Universitas Airlangga. All patients were recruited following informed written consent.

Blood Samples

Samples were collected into tubes. Serum from patients was obtained after centrifugation for 10 minutes at 10,000

rpm. Then the samples were stored and frozen at -80°C until used.

Laboratory Diagnosis

Dengue serum NS1 were detected using Panbio rapid test (immunochromatography) from Panbio Dengue Early Rapid Test with positive and negative qualitative results. NF-κB levels were measured using the Human NF-κB p65 Sandwich-ELISA kit from Elabscience followed procedures in the protocol. This is a commercial enzyme-linked immunosorbent assay for detecting NF-κB level against dengue virus in human serum or plasma.[11,12]

Statistical Analysis

In this study, were we analyzed the levels of NF-κB and NS1 in patients with dengue fever are determined clinically. We are use the Mann-Whitney test to see the difference between NS1 positive and NS1 negative in patients with dengue fever. Statistic Package for Social Sciences (SPSS) was used for data entry, processing and statistical analysis at the end of the study. P-values less than 0.05 were considered significant.

RESULT AND DISCUSSION

Study Population

A total of 40 patients suffering from dengue fever and fulfilling the inclusion criteria were enrolled in the study. Serum NS1 examination results from 40 patients found 30 patients with positive NS1 and 10 NS1 patients negative. As a control, 10 healthy people without dengue infection with various ages.

Table 1. Age and gender distribution of the participants (n=50)

	Control (n=10)/%	NS1 positive (n= 30)/%	NS1 negative (n=10)/%	Total /%
Age (mean±SD)				4.66±1.722
Age group				
0-5	0/ 0	1/ 3.3	0/ 0	1/ 2.0
6-11	0/ 0	2/ 6.7	1/ 10	3/ 6.0
12-16	1/ 10	5/ 16.7	0/ 0	6/ 12.0
17-25	8/80	8/ 26.7	3/ 30	19/ 38.0
26-35	1/ 10	5/ 16.7	1/ 10	7/ 14.0
36-45	0/ 0	4/ 13.3	2/ 20	6/ 12.0
46-55	0/ 0	2/ 6.7	2/ 20	4/ 8.0
56-65	0/ 0	2/ 6.7	1/ 10	3/ 6.0
>65	0/ 0	1/ 3.3	0/ 0	1/ 2.0
Total	10/100	30/ 100	10/ 100	50/ 100
Gender				
Male	3/30	13/14.3	6/60	22/44
Female	7/70	17/56.7	4/40	28/56
Total	10/100	30/100	10/100	50/100

Table 1 gives the age and gender distribution of the participants. The age grouping used was based on the Ministry of Health (2009) (mean 4.66 and SD = 1.722). Majority of the dengue fever were between the age group of 17 to 25 years (38.0%), the lowest were toddlers (0-4 years) and elderly (> 65 years old), namely 3.3%. There were 28 (56.0%) males and 22 (44.0%) females with various age, the females was found more than males.

NS1 Serum

NS1 was examined in the serum of dengue-infected patients collected from days 1-4 of the onset of fever and also in healthy controls. The method used for NS1 examination uses the Panbio Rapid Test Of the 40 serum samples of dengue fever patients, 30 NS1 samples were positive and 10 NS1 samples were negative. In 10 controls NS1 was negative.

Examination the levels of NF-κB

NF-κB levels were determined in serum samples in dengue fever patients collected from fever patients day 1 to 4 onsets of fever.

The level of NF-κB in serum from negative control is very low (Table 2). NF-κB levels in serum from dengue fever patients were found to be high (>10 ng/mL); higher than the NF-κB level of control in the negative control. However, the average emission in the group of patients with negative NS1 (mean=13.165) was found to be higher compared to patients with NS1 positive (mean=10.013), but there were no significant differences in NF-κB levels from 40 patients (Figure 1). In the negative control sample, there was low NF-κB level with mean value of 1.646. NF-κB levels tend to increase linearly in patients of all clinical values when the disease develops, but there is no significant difference between cases of dengue fever with NS1 positive and NS1 negative (p=0.187) (ρ>0.05).

Table 2. NF-κB in dengue fever patient

	Control (n=10)/%	NS1 positive (n= 30)/%	NS1 negative (n=10)/%	Total/%
NF-κB (mean ± SD)	1.646 ± 1.294	10.013 ± 4.808	13.165 ± 5.805	9.002±5.919
0,1–5,0 ng/mL	10/100	2/6.7	0/0	12/24
5,1–10 ng/mL	0/0	14/46.7	3/30	17/34
>10 ng/mL	0/0	14/46.7	7/70	21/42
Total	10/100	30/100	10/100	50/100

The average emission value of NF-κB levels in positive NS1 was found to be higher than in the negative NS1 group. The highest NF-κB levels were found to reach 25000 ng/mL. In the box plot the control group is at the minimum value of the dengue fever group.

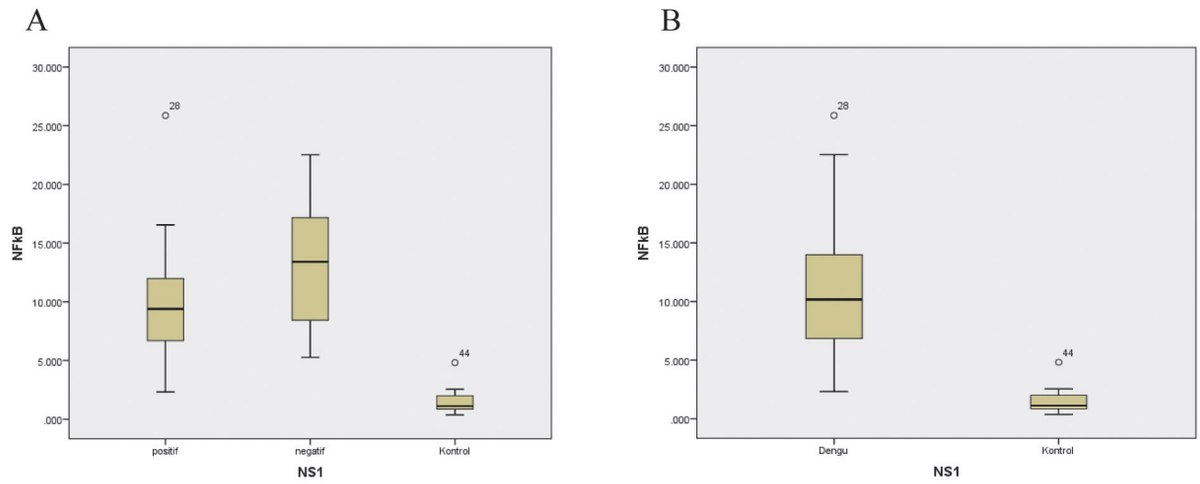

Figure 1. (A) NF-κB levels in serum from patients with dengue fever (n = 30) were determined clinically and in control (n = 10). (B) The mean value of NF-κB levels in patients with dengue fever NS1 is positive and NS1 is negative

DISCUSSION

Our results were showed that in patients with dengue fever did not always show positive results on NS1 examination in serum, this was clear in this study of 40 samples of dengue fever patients found 30 patients with NS1 positive results and 10 patients with NS1 negative. The results in NF-κB showed that the mean levels in serum of dengue fever patients were higher compared to controls.

Homogeneity of the sample in this study was carried out by limiting the duration of fever patients with days 1-4 fever. This restriction is based on a theory which states that in dengue fever patients will experience a fever phase for 2-7 days and on days 1-4 is the first fever phase where fever will appear high enough to 40°C and is the time well in the diagnosis of dengue fever where the antigen from the dengue virus can be detected at a higher level in the acute phase or in the first eight days.[13,14]

Dengue diagnosis is difficult to enforce at the beginning of the disease because the signs and symptoms are not specific so it is often difficult to distinguish from influenza virus infection, measles and typhoid fever. Viremia or the dengue virus in the bloodstream will last for 1 week. NS1 examination is an examination that detects the body parts of the virus and does not wait for the body's response to infection, so the best time to do the best examination is day 0 to day 4 and can be detected before the decline in platelets.[13] In our study there were several patients with negative NS1 results when examined using a Panbio rapid test. In, patients with dengue fever 1-4 days with NS1 positive results show that there is a dengue infection in the body. Conversely, patients dengue fever with NS1 negative do not close the possibility of dengue infection but NS1 is detected at low levels causing false negative and further examination is needed. To detect the viral protein is needed sufficient levels of the amount of virus circulating, while in the initial phase there are not enough viruses, but if it takes the sample after the appearance of antibodies the level of dengue virus will also decrease.[15]

Dengue virus (DENV) has 4 serotypes DENV-1, DENV-2, DENV-3, and DENV-4. This can cause the emergence of negative results on the examination of NS1 in patients with dengue fever is suspected to be associated with the dengue virus serotype that infects. It recommends that NS1 examination must be repeated on the seventh day later to provide more valid results.[16] NS1 is associated with the duration of the disease and is less sensitive to illness that lasts over 3 days or the level detected will decrease. They associate NS1 with the severity of the disease and the risk of progression leading to dengue hemorrhagic fever (DHF).[6] NS1 is a glycoprotein secreted by cells infected with the dengue virus, mainly in the serum supernatant of infected patients, but not in the virus.[17] NS1 is found in primary and secondary infections and can be detected in the first eight days of fever and levels will be high at the start of fever and decrease before defervescence and become negative in some cases. In Tambunan et al. they found that NS1 antigen sensitivity was higher on the third day of fever.[18] Variable antigen levels were showed that NS1 secretion is not persistent (stable). The results of research by Blacksell and Dussart stated that NS1 which binds to soluble endothelium (soluble) and collected in the hepatocyte causes circulating NS1 secretion of blood to become slow.[19,20] This can affect the level of NS1 detected in the blood.[14] NS1 is found in high concentrations in patients with severe degrees.[17]

As a reaction from the host to a viral infection that occurs in the body is the activation of one of the transcription factors namely NF-κB which will secrete various cytokines and chemokine which play a role in infection. The activation of NF-κB is triggered by the genome and protein of the virus which induces the NF-κB signaling pathway recognized by the host PRR through TLR3, TLR7, TLR8 which then activates MyD88 and IRAK-4 which subsequently leads to IRAK-1 bonding with TRAF- 6 and lead to phosphorylation

and degradation of IkB and cause translocation of NF-κB to the nucleus.[21] Activation of NF-κB will cause the secretion of proinflammatory cytokines such as IL-1, IL-6, TNF-α.

Under normal conditions without infection, NF-κB is bound to the IκB in various cells and is inactive.[22] In the event of NF-κB stimulation will be activated and detached from IκB. Because of protein exposure and viral genome NF-κB activation can also trigger extrinsic apoptotic pathways directly or with their products and cause endothelial cell death which results in bleeding. This is consistent with the results of Lin et al. an experimental study using mice and they found dengue virus protease induce cell apoptosis through its interaction with IκBα, IκBβ and cause the development of bleeding.[23]

Dengue viral protease was found to cleave IκBα and IκBβ, activating IKK and triggering activation of NF-κB which causes caspase-mediated endothelial cell apoptosis. Activation of NF-κB usually leads to the production of antiapoptotic proteins and protected cells from apoptosis. In dengue infection, NF-κB has played a pro-apoptotic role. The possibility of NF-κB-mediated endothelial apoptosis is triggered by dengue virus proteases through increased recruitment of macrophages and through increased sensitivity of endothelial cells to TNF by expressing TNF receptors.[23] However, the underlying mechanism activation of NF-κB caused by infection with the dengue virus or viral protein remains were not defined yet.

In Yi-Lin Cheng et al. states that there is an increase in NF-κB activation found in hepatomas induced by dengue virus. Research using RAW264.7 cells is showed that dengue virus protein is needed for NF-κB activation found in endothelial cells and cells undergo apoptosis within 48 hours after infection. In addition, they were found two pathways that could increase NF-κB activation in dengue infection through excessive TNF-α production and through host PRR, TLR3.[24]

Based on the results of data analysis were showed that the levels of NF-κB in dengue fever patients were higher than those of healthy people. This shows that patients with dengue fever experience more NF-κB activation than healthy people. This occurs because of the large presence of dengue viruses that can activate NF-κB which plays a role in innate defenses which then secrete various proinflammatory cytokines and chemokine that function to fight dengue virus and lead to activation of the humoral immune response. In addition, it was found that patients with negative NS1 had higher NF-kB levels than positive NS1, but did not have a significant difference. In patients who showed negative NS1 results, it did not rule out the possibility that dengue infection was occurring in the body, but the number of dengue viruses and NS1 levels in the blood was low, so that there was no detection and further examination was needed. This is not uncommon in some case and the virus can directly exploit the immune system by activating NF-κB and replicating. In this study, 13 cases of patients who died because of dengue infection, it was showed that neither dengue NS1 or dengue NS1 antibodies were detected in the endothelium, questioning the role of NS1 antibodies in the pathogenesis of acute dengue.[25]

Adikari et al. showed that acute NS1 infection was found to correlate with serum IL-10 levels and that NS1 contributed to the pathogenesis of dengue infection by inducing the production of immunosuppressive cytokines from primary monocytes.[26] Based on this, that high amounts of NS1 can induce the production of anti-inflammatory cytokines and vice versa, proinflammatory cytokines which result in an increase in the number of dengue virus replication. But how the NF-κB pathway in the pathogenesis of dengue is still unclear.

CONCLUSION

In conclusion, this study was analyzed the picture of serum NF-κB and NS1 in dengue patients where there was an increase in NF-κB activation and this is known from the high levels of NF-κB detected in serum which is thought to occur due to host cell apoptosis. And NS1 as a marker of early diagnosis of dengue infection that has been widely used was showed good results in the initial infection. But how the path of activation and involvement of NF-κB is still need further research.

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