Some epitopes conservation in non structural 3 protein dengue virus serotype 4

Tegar A. P. Siregar,[1,2] T. Mirawati Sudiro,[1] Whinie Lestari,[3] Beti Ernawati Dewi[1]

[1]Department of Microbiology, Faculty of Medicine, Universitas Indonesia/Cipto Mangunkusumo General Hospital, Jakarta
[2]Department of Microbiology, Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara, Medan
[3]Center for Biomedical and Basic Health Technology, National Institute of Health Research and Development, Ministry of Health, Jakarta

Corresponding address: dr. Tegar Adriansyah Putra Siregar, M.Biomed
E-mail: tegaradriansyah@gmail.com

Received: October 1, 2015; Revised: October 20, 2015; Accepted: October 24, 2015

Abstrak

Latar belakang: *Protein Non Struktural 3 (NS3) virus dengue menginduksi respon antibodi netralisasi dan respon sel T CD4$^+$ dan CD8$^+$, serta berperan dalam replikasi virus. Protein NS3 memiliki epitop-epitop sel T dan B yang terdapat perbedaan kelestarian pada berbagai strain virus dengue serotipe 4 (DENV-4). Penelitian ini bertujuan untuk mengetahui kelestarian epitop sel T dan B pada protein NS3 DENV-4 strain-strain dunia dan keempat serotipe virus dengue strain Indonesia.*

Metode: *Penelitian ini dilakukan di Departemen Mikrobiologi Fakultas Kedokteran UI sejak Juni 2013 - April 2014. Sekuens asam amino NS3 DENV-4 strain 081 didapatkan setelah produk PCR gen NS3 DENV-4 081 disekuensing. Epitop-epitop sel T dan sel B protein NS3 DENV-4 081 dianalisis dan dibandingkan dengan sekuens asam amino protein NS3 dari 124 strain DENV-4 di dunia dan keempat serotipe DENV strain Indonesia. Strain-strain dunia merupakan strain yang ada di benua Amerika (Venezuela, Colombia, dll) dan Asia (Cina, Singapura, dll). Referensi posisi epitop sel T dan B protein NS3 diperoleh dari laporan penelitian terdahulu.*

Hasil: *Delapan epitop sel T dan 2 epitop sel B dari protein NS3 DENV-4 081 ternyata identik dan lestari pada protein NS3 dari 124 strain DENV-4 dunia. Epitop sel B di posisi asam amino 537-544 pada protein NS3 DENV-4 081 ternyata identik dan lestari dengan epitop sel B protein NS3 dari keempat serotipe DENV strain Indonesia.*

Kesimpulan: *Kelestarian yang luas dari epitop sel T dan B pada hampir seluruh strain DENV-4 dunia dan serotipe-serotipe DENV strain Indonesia. (**Health Science Journal of Indonesia 2015;6:126-31)***

Kata kunci: *virus dengue, protein NS3, epitop sel T, epitop sel B*

Abstract

Background: Non Structural 3 (NS3) protein of dengue virus (DENV) is known to induce antibody, CD4$^+$ and CD8$^+$ T cell responses, and playing role in viral replication. NS3 protein has T and B cell epitopes, which has conservation difference between DENV-4 strains. This study aimed to identify conservation of T and B cell epitope in NS3 protein among DENV-4 strains and four serotypes DENV of Indonesia strains.

Methods: Research was held at the Department of Microbiology, Faculty of Medicine, Universitas Indonesia, June 2013 to April 2014. NS3 amino acid sequence of DENV-4 081 strain was obtained after NS3 gene of DENV-4 081 PCR products were sequenced. T and B cell epitopes of NS3 protein of DENV-4 081 strain were analysed and compared to NS3 proteins of 124 DENV-4 strains around the world and four serotypes of Indonesia strains. World strains were isolated from America (i.e. Venezuela, Colombia, etc.) and Asia (i.e. China, Singapore, etc.). For the comparison, T and B cell epitope positions of NS3 protein were obtained from published report.

Results: Eight positions of T cell epitopes and two positions of B cell epitopes of NS3 DENV-4 081 were identical and conserved to NS3 protein of 124 DENV-4 strains around the world. B cell epitope of NS3 DENV-4 081 protein at aa 537-544 was found identical and conserved to four serotypes DENV of Indonesia strains.

Conclusion: This wide conservation of T and B epitopes in almost all DENV-4 strains around the world and all serotypes of Indonesia strains. ***(Health Science Journal of Indonesia 2015;6:126-31)***

Keywords: dengue virus, NS3 protein, T cell epitope, B cell epitope

Dengue virus (DENV) infection remains a public health problem, either in the world or in Indonesia. According to data from World Health Organization (WHO), 50 to 100 million infections were estimated to occur each year, including 500,000 cases of dengue fever and 22,000 deaths, mostly among children.[1] Based on data from Indonesian Ministry of Health, the number of patients with DHF increased annually, in 2013 there were 112,511 of DHF cases in 32 provinces (incidence rate of 45.85 per 100,000 population).[2]

The virus has three structural proteins, the capsid protein (C), envelope (E) and membrane (M). These structural proteins along with seven non-structural proteins (NS) are encoded by the viral genome in the form of single-stranded RNA with positive polarity. DENV genome is a sequence of 5 'UTR-C-PRM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3' UTR.[3]

NS3 protein plays an important role in DENV life cycle.[4,5] The functions of NS3 includes a viral protease that plays a role in cutting the DENV polyprotein after translation, and plays a role in viral RNA replication through the activities as nucleotide triphosphatase (NTPase), RNA 5' triphosphatase (RTPase) and helicase.[3,6]

Non-Structural (NS) 3 protein has the ability to induce antibody responses,[7] $CD4^+$ and $CD8^+$ T cells. T cells activated by NS3 can cross-react between serotypes.[8] Anti-NS3 antibodies were proven in vivo to protect and delay the time of death (survival time) of mice challenged with DENV at doses of 100 LD_{50}.[9] NS3 sized 618 amino acid/ 69 kD, is the second largest gene/protein of DENV and its primary amino acid sequence is the most conserved.

This study aimed to obtain information about the similarity of T cell and B cell epitopes of NS3 DENV-4 081 Indonesian strain in comparison to DENV-4 world strains, as well as the similarity to the NS3 of the other serotypes Indonesia strains.

METHODS

The research was held in Department of Microbiology, Faculty of Medicine, Universitas Indonesia from June 2013 to April 2014. DENV-4 strain 081 was isolated in year 2013, a collection of National Institute of Health Research and Development, Ministry of Health, Indonesia. To get DNA sequence from DENV-4 strain 081, first viral RNA was extracted from culture supernatants of infected-C6/36 cell using QIAamp viral RNA extraction kit according to the manufacturer`s instruction (Qiagen, Hilden, Germany). Complementary DNA (cDNA) strands were reverse-transcribed using Super Script II First Strand Synthesis System with Random hexanucleotide primer according to the manufacturer`s instructions (Invitrogen, Massachusetts, USA). The entire NS3 gene was then amplified by polymerase chain reaction (PCR) using Platinum Taq Polymerase (Invitrogen, Massachusetts, USA). After purification of the PCR products using QIAquick PCR purification Kits (Qiagen GmbH, Hilden, Germany), the samples were sent to PT Genetika Science Indonesia for direct sequencing. The sequencing processes used Sanger method with Taq Big Dye Deoxy Terminator Cycle sequencing kits (Applied Biosystems, Foster City, CA USA). Nucleotide and amino acid sequence analysis was performed using GENETIC MAC- and BLAST program. Codon positions described in this study were based on the data of DENV-4 Singapore strain (GenBank accession no.GQ398256.1). Primer sequences for DNA synthesis and sequence were designed using PRIMER program and obtained from the published data of DENV4 Singapore strain (GenBank accession no.GQ398256.1).

Analysis of T and B cell epitope on NS3 protein were done by comparing the amino acid sequence of NS3 protein DENV4 081 to the NS3 protein of DENV-4 world strains and serotypes of Indonesia strains which obtained from the Genbank. Total world strains were 124 strains which 25 strains were from Asia (China, Singapore, India, Malaysia, Cambodia, India, Thailand, Philippines, Pakistan, and Taiwan), 99 strains were from America (Venezuela, Dominica, Colombia, US, Haiti, and Brazil) isolated between 1961 and 2011; and twelve Indonesian strains. The references for analyzing NS3 T [8, 10-17] and B cell epitopes [18-20] were listed in Table 1. NS3 protein epitopes of DENV-4 081, 124 DENV-4 world strains and other serotypes of Indonesia strains were analysed using Bioedit version 7.0.5.3 year 2005 (http://www.mbio.ncsu.edu/bioedit/bioedit.html).

RESULTS

Homology analysis of T cell epitopes of NS3 protein of DENV-4 081 to the NS3 protein of 124 DENV-4 world strains (25 strains from Asia and 99 strains from America) was shown in Table 2.

From fourteen T cell epitopes of the NS3 protein of DENV-4 081 compared to NS3 protein of 124 DENV-4 world strains, different amino acid at six epitopes were found in nine strains (Table 2), while the other eight epitopes were well conserved in all 124 world strains. T cell epitopes that have different amino acids were at aa 45-59, aa 145-159, aa 231-245, aa 495-509, aa 526-540 and aa 596-610 (Table 2).

Table 1. B and T cell epitopes in NS3 DENV-4 protein

No	NS3 epitope position (AA)	Reference strains	AA sequence	Reference
	T cell epitope			
1	45-59	DENV2 16681 Vietnam strain	TFHTMWHVTRGAVLM	10
2	145-159	DENV2 16681 strain	KVVGLYGNGVVTRSG	10
3	221-235	DENV3 CH53489 strain	LAPTRVVAAEMEEAL	11, 12
4	231-245	DENV2 16681 strain	MEEALRGLPIRYQTP	10
5	250-264	DENV2 16681 strain	EHTGREIVDLMCHAT	10
6	251-265	DENV4 814669 strain	HTGREIVDLMCHATF	13, 14
7	255-269	DENV2 16681strain	EIVDLMCHATFTMRL	10
8	258-272	DENV4 814669strain	DLMCHATFTTRLLSS	14
9	275-289	DENV2 16681 strain	VPNYNLIIMDEAHFT	10
10	296-310	DENV4 814669 strain	ARGYISTRVEMGEAA	4, 15
11	336-350	DENV2 2005 Singapore strain	EREIPERSWNSGHEW	16
12	495-509	DENV2 16681 Vietnam strain	LDNINTPEGIIPSMF	10
13	526-540	-	RGEQRKTFVELMRRG	17
14	596-610	DENV22005 Singapore strain	LDARIYSDPLALKEF	16
	B cell epitopes			
1	425-432	DENV3	PRRCLKPV	18
2	460-469	DENV2 Tr1751 strain	RVGRNPKNEN	19
3	521-532	DENV2 Eden 3295 strain	DETPMRGETRKV	20
4	537-544	DENV3	MRRGDLPV	18

Table 2. T and B cell epitopes analysis of NS3 DENV4 protein. Bold letters show amino acid mutation

No	NS3 Epitope position	World strains	Amino acid sequence	
			World strain	081 strain
	T cell epitopes			
1	45-59	DENV4 Cina 2010 (JF741967)	VFHTVGHVTRGSVIC	VFHTMWHVTRGSVIC
2	145-159	DENV4 India 1961 (JF262783)	RVIGLYGNGIVTKSG	KVIGLYGNGVVTKSG
3	231-245	DENV4 Venezuela 2007 (GQ199876)	MEEALRGLPVRYQTP	MEEALRGLPIRYQTP
4	495-509	DENV4 Pakistan 2009 (KF041260)	LDNIHTPEGIIPTLF	LDNIYTPEGIIPTLF
5	526-540	DENV4 Singapore 2005 (GQ398256)	RGEQRKTFVELMKRG	RGEQRKTFVELMRRG
		DENV4 Cina 2012 (KC333651)	RGEQRKTFVELMKRG	RGEQRKTFVELMRRG
		DENV4 Thailand 1997 (AY618988)	RGEQRKTFVELMKRG	RGEQRKTFVELMRRG
		DENV4 Thailand 1997 (AY618989)	RGEQRKTFVELMKRG	RGEQRKTFVELMRRG
6	596-610	DENV4 Colombia 2004 (GQ868583)	LDARVYADPMALQDF	LDARVYADPMALKDF
		DENV4 Pakistan 2009 (KF041260)	LDARVYADPVALKDF	LDARVYADPMALKDF
	B cell epitopes			
1	460-469	DENV4 Venezuela 2007 (EU854300)	RIGRNLAQED	RIGRN**P**AQED
		DENV4 Puerto Rico 1998 (FJ024424)	RIGRNPTQED	RIGRNP**A**QED
		DENV4 Venezuela 2007 (FJ882581)	RIGRNLAQED	RIGRN**P**AQED
		DENV4 Venezuela 2007 (FJ882582)	RIGRNLAQED	RIGRN**P**AQED
		DENV4 Venezuela 2007 (FJ882588)	RIGRNLAQED	RIGRN**P**AQED
		DENV4 Puerto Rico 1999 (FJ882599)	RIGRNPTQED	RIGRNP**A**QED
		DENV4 Haiti 1994 (JF262782)	RIGRNLAQED	RIGRN**P**AQED
4	537-544	DENV4 Singapore 2005 (GQ398256)	MKRGDLPV	M**R**RGDLPV
		DENV4 China 2012 (KC333651)	MKRGDLPV	M**R**RGDLPV
		DENV4 Thailand 1997 (AY618988)	MKRGDLPV	M**R**RGDLPV
		DENV4 Thailand 1997 (AY618989)	MKRGDLPV	M**R**RGDLPV

From analysis of four B cell epitopes of the NS3 protein of DENV-4 081 and 124 DENV-4 world strains, different amino acid at two epitopes were found in several strains of 124 DENV-4 world strains, i.e. aa 460 to 469 and aa 537 to 544 (Table 2). The other two B cell epitopes were well conserved in all 124 DENV-4 world strains. However, B cell epitope at aa 537 to 544 was well conserved in all four serotypes of Indonesia strains.

The T and B cell epitopes in the NS3 DENV-4 081 protein were compared to NS3 protein of DENV 1, 2, 3, and 4 Indonesia strains to identified similarity between T and B cell epitopes of NS3 protein DENV-4 and four DENV serotypes. The T cell epitopes of NS3 protein DENV-4 081 at aa 221-235, aa 231-245 and aa 296-310 were found 100% identical to the other NS3 protein DENV-4 Indonesia strains and DENV-2 Indonesia strains. The B cell epitope of NS3 protein DENV-4 081 at aa 425-432 was found 100% identical to the NS3 protein of DENV 1, 3 and 4 Indonesia strains. From all B cell epitopes, the epitope at position 537-544 was found conserved and identical in all serotypes of DENV Indonesia strains.

The characteristics of different amino acids in the six T cell epitopes which showed variability in DENV-4 081 and several 124 DENV-4 strains (Table 2) were identified by their hydrophobicity. The result showed that only at aa 145-159, aa 526-540 and aa 596-610 of NS3 protein different property of amino acid were found (Table 3), i.e. T cell epitopes of NS3 DENV-4 081 at aa 526-540 have arginine which is hydrophilic, compared to the same epitope positions of several DENV-4 strains (Table 2) have lysine which is hydrophobic. On the other T cell epitope of NS3 DENV-4 081 at aa 596-610 compared to several DENV-4 world strains there is amino acid changing from lysine (hydrophobic) to glutamine (hydrophilic).

Analysis of B cell epitopes of NS3 DENV-4 081 at aa 460-469 and aa 537-544 and several DENV-4 strains (Table 2) showed different properties of amino acids as shown in Table 3.

The B cell epitope at aa 460-469 of NS3 protein DENV-4 081 have proline which is hydrophilic, compared to the same epitope position of several DENV-4 world strains (Table 2) have leucine which is hydrophobic. The B cell epitope of NS3 protein DENV-4 081 at aa 537-544 have arginine (hydrophilic) compared to several 124 DENV-4 world strains, there is amino acid changing from arginine (hydrophilic) to lysine (hydrophobic).

DISCUSSION

From total fourteen T cell epitopes analysed, eight T cell epitopes were 100% identic and conserved to NS3 protein of 124 DENV-4 world strains (Table 2). Two of four B cell epitopes were well also conserved in 124 DENV-4 strains around the world (Table 2). This analysis also showed that from total 25 strains from Asia and 99 strains from America, 68% of Asia strains and 98% of America strains have similarity in all fourteen T cell epitopes of NS3 protein DENV-4. In total, 84% of Asia strains and 93% of America

Table 3. Hydrophobicity of amino acids in T and B cell epitopes NS3 DENV4 081 protein compared to the NS3 protein of 124 DENV4 strains

No	NS3 epitope position	AA change	Characteristics
	T cell epitopes		
1	45-59	Met → Val	No Change, both are hydrophobic
		Trp→Gly	No Change, both are hydrophobic
2	145-159	Lys →Arg	Lys →hydrophobic, Arg→hydrophilic
		Val → Ile	No Change, both are hydrophobic
3	231-245	Ile → Val	No Change, both are hydrophobic
4	495-509	Tyr → His	No Change, both are hydrophobic
5	526-540	Arg→ Lys	Lys →hydrophobic, Arg→hydrophilic
6	596-610	Met → Val	No Change, both are hydrophobic
		Lys →Gln	Lys→hydrophobic, Gln→hydrophilic
	B cell epitopes		
1	460-469	Pro →Leu	Pro →hydrophilic, Leu→hydrophobic
		Ala→Thr	No Change, both are hydrophobic
2	537-544	Arg→ Lys	Arg→hydrophilic, Lys →hydrophobic

strains showed similarity in all four B cell epitopes of NS3 protein DENV-4. From total 25 DENV-4 strains from Asia, there were eight strains showed genetic variation in the amino acid sequence of NS3 protein. The 68% similarity in all fourteen epitopes analysed in DENV-4 strains from Asia needs further study, because only 25 DENV-4 strains were obtained of total 124 world strains. The variations in the amino acid sequence of T cell epitopes of NS3 protein DENV-4 were about one or two amino acid difference (Table 2 and Table 3).

It is known that the amino acid changes would not alter the preservation area epitope recognition by antibodies and lymphocytes as long as it still have same tertiary structure. Tertiary structure of a protein is determined by various factors, one of it is hydrophobic interaction of the protein-building amino acids. Amino acid changes can cause conformational change of the protein, which may change epitope recognition by antibodies. Mutated amino acids of T cell and B cell epitopes affect hydrophobicity of epitopes so further analysis is required to get three-dimensional structure of NS3 protein.

T cell epitopes position 221-235, 231-245, and 296-310 on NS3 DENV-4 081 protein are identical to DENV-2 Indonesia strains (AY858036, GQ398268, AY858035) (Table 4). B cell epitope position 425-432 on NS3 DENV-4 081 protein is identical to DENV-1 Indonesia strains (AB189120, AB189121) and DENV-3 Indonesia strains (AY858048, AY858047, AY858046, AY858045). It is known that only one B cell epitope position 537-544 (MRRGDLPV) on the NS3 DENV-4 081 protein were identical to the four DENV serotypes Indonesia strains (Table 4). This conserve epitopes of NS3 protein DENV-4 081 to NS3 protein of all serotypes Indonesia strains were made the opportunity for the T cell and antibody produced B cell to cross recognizing between DENV serotypes.

Most T and B cell epitopes of NS3 protein (Table 1) were located in the NS3 helicase, only two of the T cell epitopes from total fourteen T and B cell epitopes were located in the NS3 protease. Helicase has three subdomains with significant sequence identity and structural similarity with helicase of another flavivirus.[21] NS3 helicase has RNA unwinding activity by disrupting the hydrogen bonds of double strands DNA.

In conclusion, this wide conservation of T and B epitopes were found in almost all DENV-4 strains around the world and all serotypes of Indonesia strains.

Table 4. T dan B cell epitopes position of NS3 DENV4 081 protein which is identical to NS3 DENV 1, 2, 3 dan 4 protein Indonesia strain

No	T cell epitope position	B cell epitope position	AA epitope sequence of DENV4 081 strain	DENV1	DENV2	DENV3
1	221-235		LAPTRVVAAEMEEAL		Identical	
2	231-245		MEEALRGLPIRYQTP		Identical	
3	296-310		ARGYISTRVEMGEAA		Identical	
4		425-432	PRRCLKPV	Identical		Identical
5		537-544	MRRGDLPV	Identical	Identical	Identical

Acknowledgments

This study and publication supported by National Institute of Health Research and Development Grant year 2013, Ministry of Health, Indonesia.

REFFERENCES

1. WHO. Impact of dengue [Cited 2015]. Available from: http://www.who.int/csr/disease/dengue/impact/en/#content
2. Directorate General of Disease Control and Environmental Sanitation, Ministry of Health, Indonesia. Diseases caused by mosquitoes and how to prevent and targets to be achieved by the government [cited 2014 April 15]. Available from: http://pppl.depkes.go.id/focus?id=1374. Indonesian.
3. Beasley DW, Barrett AD. The infectious agent. In: Halstead SB, editor. Dengue. London: Imperial College Press; 2008. p. 29-73.
4. Li J, Lim SP, Beer D, et al. Functional profiling of recombinant NS3 proteases from all four serotypes of dengue virus using tetrapeptide and octapeptide substrate libraries. J Biol Chem. 2005;280:28766-74.
5. Patkar CG, Kuhn RJ. Yellow fever virus NS3 plays an essential role in virus assembly independent of its known enzymatic functions. J Virol. 2008;82(7):3342-52.
6. Gubler DJ. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st Century. Trends Microbiol. 2002;10:100–3.

7. Chen Z, Tian Y, Liu L, An J. Production of a monoclonal antibody against non-structural protein 3 of dengue-2 virus by intrasplenic injection. Hybridoma. 2008;27(6):467-71.
8. Spaulding AC, Kurane I, Ennis FA, Rothman AL. Analysis of murine CD8(+) T-cell clones specific for the dengue virus NS3 protein: flavivirus cross-reactivity and influence of infecting serotype. J Virol. 1999;73:398–403.
9. Tan CHC, Yap EH, Singh M, et al. Passive protection studies in mice with monoclonal antibodies directed against the non-structural protein NS3 of dengue 1 virus. J Gen Virol. 1990;71:745-8.
10. Simmons CP, Dong T, Chau NV, et al. Early T-cell responses to dengue virus epitopes in Vietnamese adults with secondary dengue virus infections. J Virol. 2005;79:5665-75.
11. Mathew A, Kurane I, Green S, et al. Predominance of HLA-restricted cytotoxic T-lymphocyte responses to serotype-cross-reactive epitopes on nonstructural proteins following natural secondary dengue virus infection. J Virol. 1998;72:3999-4004.
12. Kurane I, Zeng L, Brinton MA, Ennis FA. Definition of an epitope on NS3 recognized by human CD4+ cytotoxic T lymphocyte clones cross-reactive for dengue virus types 2, 3, and 4. Virology. 1998;240:169-74.
13. Sanchez V, Gimenez S, Tomlinson B, et al. Innate and adaptive cellular immunity in flavivirus-naive human recipients of a live-attenuated dengue serotype 3 vaccine produced in Vero cells (VDV3). Vaccine. 2006;24(23):4914-26.
14. Okamoto Y, Kurane I, Leporati AM, et al. Definition of the region on NS3 which contains multiple epitopes recognized by dengue virus serotype-cross-reactive and flavivirus-cross-reactive, HLA-DPw2-restricted CD4+ T cell clones. J Gen Virol. 1998;79(Pt 4):697-704.
15. Rothman AL, Kurane I, Ennis FA. Multiple specificities in the murine CD4+ and CD8+ T-cell response to dengue virus. J Virol. 1996;70:6540-6.
16. Rivino L, Kumaran EA, Jovanovic V, et al. Differential targeting of viral components by CD4+ versus CD8+ T lymphocytes in dengue virus infection. J Virol. 2013;87:2693-706.
17. Imrie A, Meeks J, Gurary A, et al. Differential functional avidity of dengue virus-specific T-cell clones for variant peptides representing heterologous and previously encountered serotypes. J Virol. 2007;81:10081-91.
18. Amin N, Aguilar A, Chamacho F, et al. Identification of Dengue-specific B-Cell Epitopes by Phage-display Random Peptide Library. The Malaysian journal of medical sciences: MJMS. 2009;16:4-14.
19. Tian Y, Chen W, Yang Y, et al. Identification of B cell epitopes of dengue virus 2 NS3 protein by monoclonal antibody. Applied microbiology and biotechnology. 2013;97(4):1553-60.
20. Moreland NJ, Tay MY, Lim E, et al. High affinity human antibody fragments to dengue virus non-structural protein 3. PLoS neglected tropical diseases. 2010;4(11):e881.
21. Luo D, Xu T, Hunke C, et al. Crystal structure of the NS3 protease-helicase from dengue virus. J Virol. 2008;82:173-83.