American Journal of Infectious Diseases and Microbiology, 2017, Vol. 5, No. 2, 74-79
Available online at http://pubs.sciepub.com/ajidm/5/2/1

DOI:10.12691/ajidm-5-2-1

Thrombocytopenia in Patients with Dengue Virus Infection and Correlation between Circulating Soluble MICB Protein Level and Platelet Counts

Le Van Tuan*, Phan Thi Tuyet Nga, Trinh Thi Hong Hanh, Nguyen Thi Thu Tram, Pham Tho Duoc

Department of Virology, Tay Nguyen Institute of Hygiene and Epidemiology, Buon Ma Thuot city, DakLak, Vietnam
*Corresponding author: levantuan_tihe@yahoo.com

Abstract **Background:** Soluble MHC class I polypeptide-related sequence B (sMICB) has been associated with the development of severity of dengue fever. However, serum sMICB level and relationship with platelets in patients with dengue virus (DENV) infection remain unclear. The aims of this study are to identify soluble MICB serum level and the correlation with platelets counts in patients with dengue virus infection. **Methods:** A total of 88 patients were confirmed with an acute phase of DENV infection (1–7 days after the onset of illness) based on the result a positive reverse transcriptase-polymerase chain reaction (RT-PCR), or anti-dengue IgM antibodies were used. Serum soluble MICB level was measured by MICB ELISA. **Results:** Serum soluble MICB (sMICB) levels in dengue virus infected patients were observed a median of 146.3 pg/ml. Serum sMICB was significantly higher in dengue patients with warning signs and severe compared to patients without warning signs. However, no significant difference of sMICB between age groups of dengue patients; and between primary and secondary infection were observed (P > 0.05). The significantly negative correlation between serum sMICB levels and platelet counts was found (Spearman's rho = -0.34, P = 0.001). **Conclusion:** Serum sMICB levels might be considered as a potential prognostic biomarker for dengue patients.

Keywords: *dengue fever, dengue virus infection, soluble MHC class I polypeptide-related sequence B*

Cite This Article: Le Van Tuan, Phan Thi Tuyet Nga, Trinh Thi Hong Hanh, Nguyen Thi Thu Tram, and Pham Tho Duoc, "Thrombocytopenia in Patients with Dengue Virus Infection and Correlation between Circulating Soluble MICB Protein Level and Platelet Counts." *American Journal of Infectious Diseases and Microbiology*, vol. 5, no. 2 (2017): 74-79. doi: 10.12691/ajidm-5-2-1.

1. Introduction

Dengue fever (DF) is one of the most common mosquitos-borne viral diseases of the tropical and subtropical regions, with an estimated annually occurrence of 100 million cases [1]. Dengue virus (DENV) is single-stranded RNA viruses that belong to the family *Flaviviridae*, and the genus *Flavivirus*. Dengue infections are caused by any one of the four distinct but closely related dengue virus (DENV) serotypes (called DENV-1, DENV-2, DENV-3, and DENV-4) [2]. Dengue fever results in a wide spectrum of clinical manifestations ranging from asymptomatic or mild illness to severe dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS) which is characterized hemodynamic disturbances, increased vascular permeability, hypovolemia, hypotension, and shock [2]. Thrombocytopenia and platelet dysfunction are common in both cases and are related to the clinical outcome [3]. A genome-wide association study (GWAS) found two susceptible loci associated with DSS is MHC class I polypeptide-related sequence B (MICB) and the other is phospholipase C epsilon 1 (PLCE1) in dengue patients [4-6]. MHC class I (MHC-I) proteins encoded by the MHC-I chain-related sequence B (MICB) gene, present in natural killer (NK) cells and cytotoxic T (CD8+) cells [7], are responsible for presenting antigenic proteins of dengue virus to lymphocytes for immune modulation. The MICB single nucleotide polymorphism (SNP) rs3132468 was also associated with symptomatic dengue compared to non-dengue causes of acute febrile illnesses in children and adults, and in infants [8]. Soluble (s) MICB is produced by proteolytic cleavage of MICB on cell-surface membranes and can be measured in patient sera [9].

The aims of this study are to identify and analyze sMICB serum level and its correlation with a number of platelets in dengue patients.

2. Materials and Methods

2.1. Patients and Study Design

Blood samples were collected from dengue suspected febrile patients in Dak Lak hospital, Vietnam in 2015. Five milliliter of venous blood were collected aseptically then labeled and processed at the different diagnostic departments. The blood was centrifuged at 3000 rpm for

10 min and the serum was used for the detection of IgM and IgG antibodies against dengue virus; and RT-PCR. The platelet count was determined by automatic hemocytometer (Sysmex, Hyogo, Japan). Patients were grouped into dengue without warning signs, dengue with warning signs, and severe dengue according to the new 2009 WHO classification scheme proposed [1]. Dengue with warning signs (abdominal pain, persistent vomiting, fluid accumulation, mucosal bleeding, lethargy, liver enlargement, increasing haematocrit with decreasing platelets); and severe dengue (dengue with severe plasma leakage, severe bleeding, or organ failure). The patient data including the patient's gender, age, area of residence, admission date were obtained from records and registers/filled forms, and questionnaire.

Patients who were confirmed with an acute phase of DENV infection (1–7 days after the onset of illness) based on the result a positive reverse transcriptase-polymerase chain reaction (RT-PCR), or anti-dengue IgM antibodies were subjected for quantification of sMICB level in serum.

2.2. Enzyme-Linked ImmunoSorbent Assay for Dengue IgM and IgG antibodies

All the samples were tested for dengue IgM and IgG using Enzyme-Linked ImmunoSorbent Assay (ELISA) using commercial kit (IBL International GmbH, America). Absorbance was read using an ELISA reader (Biotex, USA) at a wavelength of 450 nm. The colour intensity is directly related to the dengue antibody concentration in each test sample.

2.3. Viral RNA Extraction and Semi-nested RT-PCR

Serum samples obtained in early phase of disease (within 5 days of illness) were tested for dengue viral RNA using RT-PCR. RNA was extracted from serum samples using QIAGEN QIAamp viral RNA mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The extracted RNA was used for the identification of the DENV serotypes using RT-PCR. The identification was carried out by RT-PCR followed by nested PCR by demonstrating the presence of virus specific RNA employing dengue group-specific as well as serotype-specific primers targeting C-prM gene junction following the protocol of Lanciotti et al. as described previously [10].

2.4. Identification of Dengue Primary and Secondary Infection

Primary or secondary dengue virus infection was defined by the ratio of the IgG to IgM readings as described by Cucunawangsih et al [11]. Primary dengue virus infection was defined if the IgG:IgM OD ratio was <1.14, or secondary infection if the OD ratio was ≥1.1.4.

2.5. Quantification of sMICB Serum Levels

Soluble MICB levels were measured in the sera of the dengue patients using a commercially available Human MICB ELISA Kit (Sigma Aldrich) following the manufacturer's instructions. Briefly, standards and samples were added into appropriate wells of coated ELISA plate and incubated over night at 4°C with gentle shaking. After plates were washed, 100 µl of biotinylated MICB detection was added to each well. After incubation at room temperature (RT), 100 µl of HRP-Streptavidin solution was added to each well and incubated at RT. Subsequently, the plates were washed again, and TMB substrate was added to each well, incubated at RT in the dark with gentle shaking. Finally, the reaction was stopped and plates were read at 450 nm immediately. The standard curve was plotted log-log graph based upon the mean of absorbance, and the best-fit straight line were drawn through the standard points. The concentrations of serum sMICB protein were calculated according to the standard curve. The minimum detectable limit of sMICB serum proteins was 0.069 pg/ml.

2.6. Statistical Analysis

Since the data were not distributed normally, a Mann–Whitney rank sum test was performed to compare values between two groups and a Kruskal-Wallis test was performed to compare values between more than two groups. Values were shown as median [Interquartile range (IQR)]. Spearsons correlation as well as linear regression was done to analyze the correlation between variables. Analyses were performed with GraphPad Prism (GraphPad Software, Inc. Version 5.0). For all statistical analyses, P values less than 0.05 were considered statistically significant.

3. Results

Sum of 282 dengue suspected subjects were enrolled in this setup. Of the 282 dengue suspected cases, 29 cases were seen positive for dengue viral RNA by RT-PCR and 59 cases were positive for dengue IgM, or both IgG and IgM by ELISA. The entire of dengue positive cases was 31.20%.

3.1. Characteristics of the Dengue Positive Patients

Of 88 dengue positive patients, a majority, 37 (42.04%) of the dengue cases was in the age group of 15 – 30 years, followed by age group of 31-50 years (36.36%). Among 88 dengue patients, 43 (48.86%) were males and 45 (51.14%) were females. The age distribution is given in Table 1. Secondary infection was predominant (66/88), accounted for 75.00% of dengue cases.

3.2. Platelet Counts of Dengue Positive Patients

Of 48 dengue patients with thrombocytopenia (platelet count less than 100,000 per mm^3), 30 patients (34.09%) had platelet counts between 51,000 and 100,000 (mild thrombocytopenia), 14 patients (15.91%) had platelet counts between 21,000 and 50,000 (moderate

thrombocytopenia), while the remaining 4 patients (4.55%) had platelet counts less than 20,000 (severe thrombocytopenia) (shown in Table 1).

Of the 88 seropositive patients, 47 (53.85%) patients clinically presented with dengue without warning signs, followed by 33 (37.50%) patients with dengue with warning signs and remaining 8 (9.09%) in severe dengue. Among the thrombocytopenia cases, it was observed that, 70.96% (22/31 cases) of patients with mild thrombocytopenia, 100% (14/14 cases) of patients with moderate thrombocytopenia and 100% (3/3) of patients with severe thrombocytopenia presented with clinical features of dengue with warning signs/severe dengue. The relationship between severity of thrombocytopenia and clinical category of dengue infection is shown in Table 2.

3.3. Serum Soluble MICB in Dengue Positive Patients

Serum soluble MICB (sMICB) levels were determined in 88 dengue virus infected patients. We observed a median of 146.3 pg/ml [102.9-349.1] in dengue patients. Serum sMICB were significantly higher in dengue patients with warning signs (median, 206.5 pg/ml) and severe (median, 686.5 pg/ml) than patients without warning signs (median, 116.2 pg/ml) (P <0.001). However, there was no significant difference of the serum sMICB between the dengue patients with warning signs and severe groups (Figure 1).

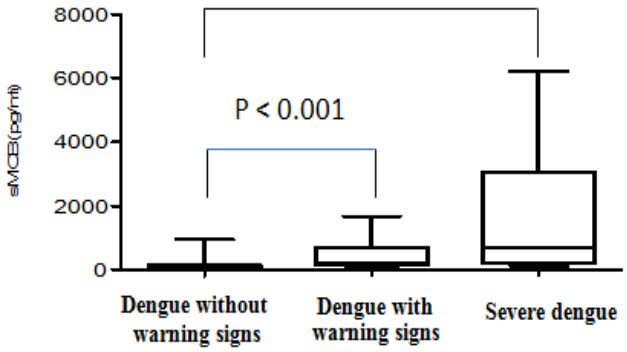

Figure 1. Serum sMICB level in dengue positive patient groups of clinical manifestations

Table 1. Age group wise distribution and platelet count of dengue positive patients

Age groups	Platelet count (per mm^3)				Total
	<20,000	21,000-50,000	51,000-100,000	>100,000	
<15 years	1 9.09%	2 18.19%	1 9.09%	7 63.63%	11 100%
15-30 years	1 2.70%	7 18.92%	15 40.54%	14 37.84%	37 100%
31-50 years	2 6.26%	3 9.37%	12 37.50%	15 46.87%	32 100%
>50 years	0 0%	2 25.00%	2 25.00%	4 50.00%	8 100%
Total	4 4.55%	14 15.91%	30 34.09%	40 45.45%	88 100%

Table 2. Platelet counts and clinical category of dengue positive patients

Platelet counts	Category of dengue			Total
	Dengue without warning signs	Dengue with warning signs	Severe dengue	
>100,000/mm^3	38 95.00%	2 5.00%	0 0%	40 100%
51,000-100,000/mm^3	9 29.03%	21 67.74%	1 3.23%	31 100%
20,000-50,000/mm^3	0 0%	10 71.43%	4 28.57%	14 100%
<20,000/mm^3	0 0%	0 0%	3 100%	3 100%
Total	47 53.41%	33 37.50%	8 9.09%	88 100%

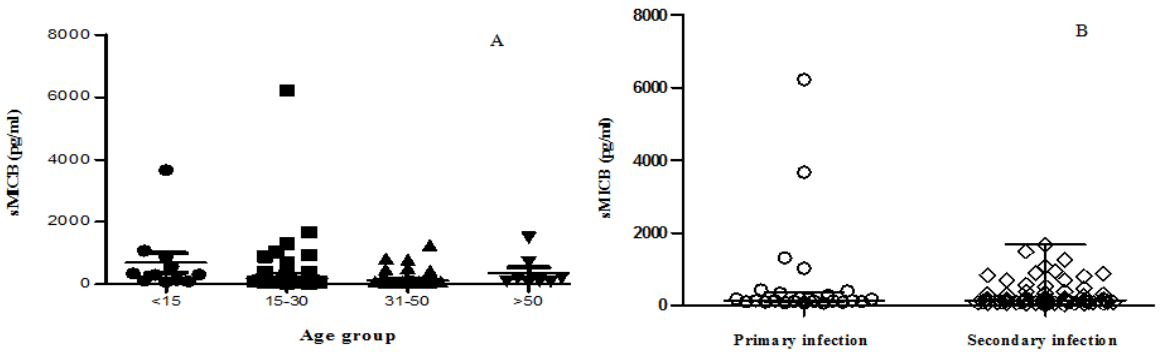

Figure 2. Serum sMICB level in age groups (A) and Serum sMICB level and type of dengue infection (B)

There is no significant difference of the serum sMICB between age groups of dengue patients; and secondary of dengue infection (P>0.05) (Figure 2).

3.4. Relationship between sMICB Serum Levels and Platelet Counts

Relationship between serum sMICB and clinical parameters was evaluated to reveal the roles of sMICB in the progression of dengue infection. In dengue patients, we observed a significantly negative correlation between serum sMICB levels and platelet counts (Spearman's rho = -0.34, P = 0.001) (Figure 3).

However, the serum sMICB level was not significantly correlated with lymphocyte counts (Figure 4) and hematocrit (Figure 5) (Spearman's rho = -0.28, P = 0.24 and Spearman's rho = -0.07, P = 0.46; respectively).

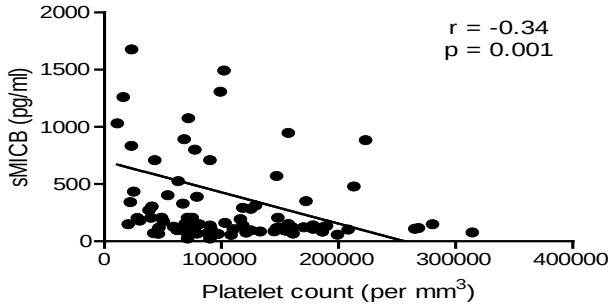

Figure 3. Correlation between serum sMICB levels and platelet counts in dengue positive patients. The sMICB serum levels were correlated with platelet count using Spearman's rank correlation coefficient test. The Spearman's rho and P value are also presented

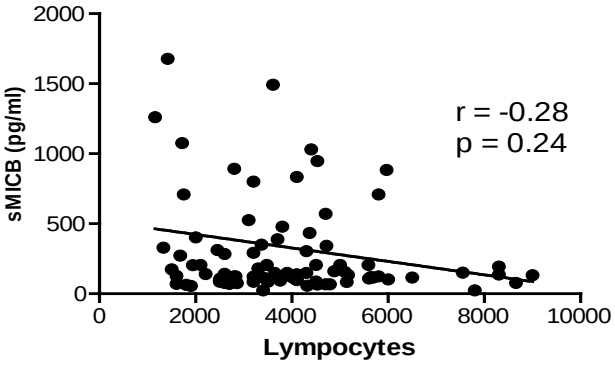

Figure 4. Correlation between serum sMICB levels and lympocytes in dengue positive patients

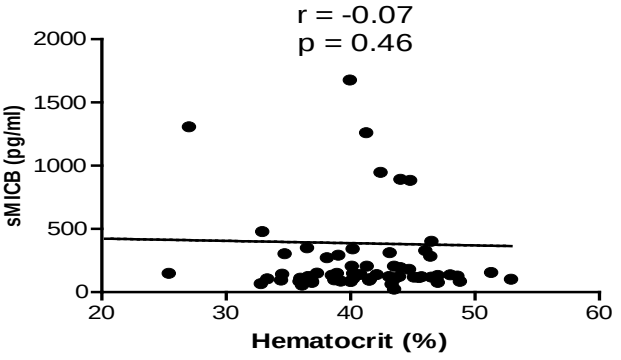

Figure 5. Correlation between serum sMICB levels and hematocrit in dengue positive patients

4. Discussion

In the present study, most patients with serological and virological confirmation were in the age group of 15-50 years and this was in accordance with the study by Khan et al., [12]. In Indonesia, data from 1968 to 2013 showed that the dengue incidence has been increasing in over 15 year olds [13], a pattern that has been observed in other high endemic Southeast Asia countries [14,15,16,17]. Although DF is a self-limited febrile illness, DHF is characterized by prominent hemorrhagic manifestations associated with thrombocytopenia and an increased vascular permeability [18].

Thrombocytopenia is one of the diagnostic criteria of DHF and DSS [19]. Though the dengue virus induced bone marrow suppression decreased platelet synthesis, an immune mechanism of thrombocytopenia caused by increased platelet destruction appears to be operative in patients with DHF [20]. The present study showed dengue with warning signs/severe dengue was more common in Dengue patients with thrombocytopenia. Previous study indicated that patients with DHF/DSS had lower platelet counts than patients with only DF [21]. The study in India, thrombocytopenia was found in 71% of dengue cases [12]. In the present study 54% of cases of dengue had thrombocytopenia.

Human major histocompatibility complex (MHC) class I polypeptide-related sequence B (MICB) is ligands of the natural killer group 2 member D(NKG2D) receptor, a stimulatory receptor on NK cells and a co-stimulatory receptor on CD8+ T-cells [22]. The interaction between NKG2D receptor and its ligand results in an activation of NK, NKT cells, CD8+ T cells and additional T cell subsets, enhancing their cytolytic ability and cytokine production during viral infection [22,23,24]. Thus, the MICB–NKG2D pathway is an important mechanism by which the host immune system recognizes and kills infected cells [25,26]. In addition to those membrane-bound forms, MICB is also cleaved proteolytically from viral infected cells and appeared as soluble forms (sMICB) in sera of patients with dengue infection. sMICB blocks activation signal generated by MICB-NKG2D interaction, and cause immune evasion strategy [9]. The present study found that sMICB concentration in sera of dengue patients with warning signs, and severe dengue is higher compared to dengue without warning signs (P <0.001). The previous studies demonstrated that sMICB could inhibit the activation of the anti-viral effect of NK cells and CD8+T cells, and thus contribute to the progression of disease severity. Recent study revealed the rs3132468-C of human MICB gene, was significantly associated with DSS due to lesser mRNA expression of MICB [5]. Dengue virus-infected cells with lower MICB expression could escape from the NKG2D-mediated killing by NK cells. Infected cells escaping the host immune response may mediate the pathogenesis of DSS because the high dengue viremia titer is associated with increased disease severity [8]. The infected cells escape from the immune response of the host could result in a higher virus titre and an increased of developing severe dengue and DSS [27]. It is generally speculated that sMICB produced from viral infected cells may deactivate NKG2D-mediated immune responses [28,29].

Thrombocytopenia has always been one of the criteria used by WHO guidelines as a potential indicator of clinical severity [30,31]. In the present study indicated that higher sMICB level has been shown to be associated with severity of the disease. Interestingly, we observed a negative correlation between serum sMICB and platelet counts in DENV infected patients. Although the mechanisms by which sMICB may participate to vascular pregnancy diseases remain unclear. This could be explained that serum sMICB from patients with dengue infection can impact NKG2D expression and modulate NK-mediated IFN-γ production [32,33].

IFN-γ is produced during a T-lymphocyte helper response type 1 and may reflect CD8+ T cell activation with production of inflammatory cytokines. High levels of IFN-γ were observed in patients with dengue from Asian and Latin America and were associated with severity [34]. IFN-γ produced by T cells may also activate mononuclear phagocytes (monocytes and dendritic cells), which would produce factors such as TNF-α, tissue factor, and platelet-activating factor, among other mediators. These factors may all participate in activation of platelet and endothelial cells, leading to platelet consumption, increase in endothelial permeability, hypotension and ultimately to shock [35,36].

Abbreviations

DENV: Dengue virus **DHF:** Dengue hemorrhagic fever **ELISA:** Enzyme-linked immunosorbent assay **RT-PCR:** Reverse transcriptase polymerase chain reaction

Ethical Statement

The study was approved by the institutional review boards of Tay Nguyen Institute of Hygiene and Epidemiology, Vietnam (No: VTN-58).

Conflicts of Interest

The authors declare that they have no relevant financial or personal conflicts of interests.

Funding

The study was partially granted by Dengue fever prevention program funded by Ministry of Health, Vietnam.

Acknowledgements

The authors also wish to thank the medical staffs of the hospitals of DakLak for the collection of the serum samples.

References

[1] WHO: Comprehensive Guidelines for Prevention and Control of Dengue and Dengue Hemorrhagic Fever. *Revised and expanded edition New Delhi, India* 2011:11-16.

[2] Martina BE, Koraka P, Osterhaus AD: Dengue virus pathogenesis: an integrated view. *Clinical microbiology reviews* 2009, 22(4): 564-581.

[3] Harris E, Videa E, Perez L, Sandoval E, Tellez Y, Perez ML, Cuadra R, Rocha J, Idiaquez W, Alonso RE *et al*: Clinical, epidemiologic, and virologic features of dengue in the 1998 epidemic in Nicaragua. *The American journal of tropical medicine and hygiene* 2000, 63(1-2): 5-11.

[4] Khor CC, Chau TN, Pang J, Davila S, Long HT, Ong RT, Dunstan SJ, Wills B, Farrar J, Van Tram T *et al*: Genome-wide association study identifies susceptibility loci for dengue shock syndrome at MICB and PLCE1. *Nat Genet* 2011, 43(11): 1139-1141.

[5] Dang TN, Naka I, Sa-Ngasang A, Anantapreecha S, Chanama S, Wichukchinda N, Sawanpanyalert P, Patarapotikul J, Tsuchiya N, Ohashi J: A replication study confirms the association of GWAS-identified SNPs at MICB and PLCE1in Thai patients with dengue shock syndrome. *BMC Medical Genetics* 2014, 15(1):1-5.

[6] Taqi MM, Waseem D, Ismatullah H, Haider SA, Faisal M: In silico transcriptional regulation and functional analysis of dengue shock syndrome associated SNPs in PLCE1 and MICB genes. 2016, 16(3):335-345.

[7] Steinle A, Li P, Morris DL, Groh V, Lanier LL, Strong RK, Spies T: Interactions of human NKG2D with its ligands MICA, MICB, and homologs of the mouse RAE-1 protein family. *Immunogenetics* 2001, 53(4):279-287.

[8] Whitehorn J, Chau TN, Nguyet NM, Kien DT, Quyen NT, Trung DT, Pang J, Wills B, Van Vinh Chau N, Farrar J *et al*: Genetic variants of MICB and PLCE1 and associations with non-severe dengue. *PloS one* 2013, 8(3):e59067.

[9] Chitadze G, Bhat J, Lettau M, Janssen O, Kabelitz D: Generation of soluble NKG2D ligands: proteolytic cleavage, exosome secretion and functional implications. *Scandinavian journal of immunology* 2013, 78(2):120-129.

[10] Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vorndam AV: Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. *J Clin Microbiol* 1992, 30(3): 545-551.

[11] Cucunawangsih, Lugito NP, Kurniawan A: Immunoglobulin G (IgG) to IgM ratio in secondary adult dengue infection using samples from early days of symptoms onset. *BMC infectious diseases* 2015, 15:276.

[12] Khan DM KK, S S, V R M: Evaluation of thrombocytopenia in dengue infection along with seasonal variation in rural melmaruvathur. *Journal of clinical and diagnostic research : JCDR* 2014, 8(1):39-42.

[13] Karyanti MR, Uiterwaal CSPM, Kusriastuti R, Hadinegoro SR, Rovers MM, Heesterbeek H, Hoes AW, Bruijning-Verhagen P: The changing incidence of Dengue Haemorrhagic Fever in Indonesia: a 45-year registry-based analysis. *BMC Infectious Diseases* 2014, 14(1):412.

[14] Gubler DJ: Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic. *Trends Microbiol* 2002, 10(2):100-103.

[15] Guha-Sapir D, Schimmer B: Dengue fever: new paradigms for a changing epidemiology. *Emerg Themes Epidemiol* 2005, 2(1):1.

[16] Cummings DA IS, Lessler JT, McDermott A, Prasanthong R, Nisalak A, Jarman RG, Burke DS, Gibbons RV.: The impact of the demographic transition on dengue in Thailand: insights from a statistical analysis and mathematical modeling. *PLoS Med* 2009, 6(9).

[17] Thai KT NH, Hoang PL, Tran NT, Phan GT, Le HQ, Tran BQ, Nguyen NV, de Vries PJ: Age-specificity of clinical dengue during primary and secondary infections. *PLoS neglected tropical diseases* 2011, 5(6).

[18] Saito M OK, Inoue S, Dimaano EM, Alera MT, Robles AM, Estrella BD Jr, Kumatori A, Moji K, Alonzo MT, Buerano CC, Matias RR, Morita K, Natividad FF, Nagatake T: Association of increased platelet-associated immunoglobulins with thrombocytopenia and the severity of disease in secondary dengue virus infections. *Clinical and experimental immunology* 2004, 138(2): 299-303.

[19] WHO: Dengue hemorrhagic fever diagnosis, treatment and control. *WHO: Geneva* 1997, 2nd edn.

[20] De Castro RA dCJ, Barez MY, Frias MV, Dixit J, Genereux M.: Thrombocytopenia associated with dengue hemorrhagic fever responds to intravenous administration of anti-D (Rh (o) -D) immune globulin. *Am J Trop Med Hyg* 2007, 76(4):737-742.

[21] Mourão MP LM, Macedo VO, Santos JB: Thrombocytopenia in patients with dengue virus infection in the Brazilian Amazon. *Platelets* 2007, 18(8): 605-612.

[22] Gonzalez S, Groh V, Spies T: Immunobiology of human NKG2D and its ligands. *Curr Top Microbiol Immunol* 2006, 298: 121-138.

[23] Bahram S: MIC genes: from genetics to biology. *Advances in immunology* 2000, 76: 1-60.

[24] Groh V, Rhinehart R, Randolph-Habecker J, Topp MS, Riddell SR, Spies T: Costimulation of CD8alphabeta T cells by NKG2D via engagement by MIC induced on virus-infected cells. *Nature immunology* 2001, 2(3):255-260.

[25] Beltran D, Lopez-Verges S: NK Cells during Dengue Disease and Their Recognition of Dengue Virus-Infected cells. *Front Immunol* 2014, 5:192.

[26] Petitdemange C, Wauquier N, Rey J, Hervier B, Leroy E, Vieillard V: Control of acute dengue virus infection by natural killer cells. *Frontiers in immunology* 2014, 5: 209.

[27] Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, Endy TP, Raengsakulrach B, Rothman AL, Ennis FA *et al*: Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *J Infect Dis* 2000, 181.

[28] Groh V, Wu J Fau - Yee C, Yee C Fau - Spies T, Spies T: Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. (0028-0836 (Print)).

[29] Salih HR, Rammensee Hg Fau - Steinle A, Steinle A: Cutting edge: down-regulation of MICA on human tumors by proteolytic shedding. *J Immunol* 2002, 169(8)(0022-1767 (Print)):4098-4102.

[30] WHO: Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control. *ISBN: 9789241547871* 2009, New edition.

[31] Jayashree K, Manasa GC, Pallavi P, Manjunath GV: Evaluation of platelets as predictive parameters in dengue Fever. *Indian journal of hematology & blood transfusion : an official journal of Indian Society of Hematology and Blood Transfusion* 2011, 27(3): 127-130.

[32] Croy BA, He H, Esadeg S, Wei Q, McCartney D, Zhang J, Borzychowski A, Ashkar AA, Black GP, Evans SS *et al*: Uterine natural killer cells: insights into their cellular and molecular biology from mouse modelling. *Reproduction (Cambridge, England)* 2003, 126(2):149-160.

[33] Lee H, Schlereth SL, Park EY, Emami-Naeini P, Chauhan SK, Dana R: A novel pro-angiogenic function for interferon-gamma-secreting natural killer cells. *Investigative ophthalmology & visual science* 2014, 55(5):2885-2892.

[34] Azeredo EL, Zagne SM, Alvarenga AR, Nogueira RM, Kubelka CF, de Oliveira-Pinto LM: Activated peripheral lymphocytes with increased expression of cell adhesion molecules and cytotoxic markers are associated with dengue fever disease. *Memorias do Instituto Oswaldo Cruz* 2006, 101(4):437-449.

[35] Mangada MM, Endy TP, Nisalak A, Chunsuttiwat S, Vaughn DW, Libraty DH, Green S, Ennis FA, Rothman AL: Dengue-specific T cell responses in peripheral blood mononuclear cells obtained prior to secondary dengue virus infections in Thai schoolchildren. *The Journal of infectious diseases* 2002, 185(12):1697-1703.

[36] Mangada MM, Rothman AL: Altered cytokine responses of dengue-specific CD4+ T cells to heterologous serotypes. *Journal of immunology (Baltimore, Md : 1950)* 2005, 175(4):2676-2683.