

# A GLANCE AT THE VON WILLEBRAND FACTOR IN DENGUE VIRUS INFECTION

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**Abstract.** To identify the level of von Willebrand factor (vWF) in dengue infection, especially severe DHF, and correlate the increase in vWF with thrombocytopenia, children admitted with dengue fever/DHF were examined for hemoglobin, hematocrit, platelet, and vWF for three consecutive days. Anti-dengue IgM and IgG were determined. Correlations between vWF and thrombocytopenia were analyzed using multivariate analysis. Forty-one patients were eligible for the study; of whom almost three fourths had a secondary infection, as proved serologically. At the beginning of the study, a high level of vWF along with a low platelet count were seen, which seemed to fit the hypothesis that an increase in vWF in the serum will be followed by a decrease in platelets, as a result of the platelet aggregation process in the peripheral blood vessels, predisposed by the immune complex events in DHF. Observations for three consecutive days revealed significant changes of vWF levels ( $p = 0.000$ ) as well as platelet counts ( $p = 0.002$ ). However in the context of dengue infections, these changes did not correlate well ( $p = 0.988$ ). Could there have been a significant correlation if cases were followed for a longer period of time? Being a part of a more comprehensive study, it appeared that in patients with dengue infections, vWF and platelets were not the only factors involved in bleeding, indicating that activation of endothelium is one factor in a multifactorial process.

## INTRODUCTION

The viral hemorrhagic fevers, *ie* dengue hemorrhagic fever (DHF), are hallmarked by bleeding complications which may be life-threatening. The pathogenesis of bleeding in DHF is poorly understood. Thrombocytopenia may enhance risk, but the primary cause of bleeding is unknown (Rigau-Perez, 1998). Limited data suggest that activation of coagulation and fibrinolysis play a role in the pathogenesis of DHF. An imbalance in the regulation of coagulation and fibrinolysis, in conjunction with thrombocytopenia may contribute to the bleeding tendency in DHF (van Gorp *et al.*, 2001). Endothelial dysfunction is usually followed by an increase in procoagulant activation, decrease in anticoagulant activation, and increase in synthesis and secretion of von Willebrand factor (vWF) as well

as its propeptide (van Gorp *et al.*, 1997).

An attempt was made to observe plasma levels of vWF in dengue hemorrhagic fever, and to see the relationship between the vWF and thrombocytopenia in dengue hemorrhagic fever. This study is part of a more comprehensive study analyzing factors involved in platelet aggregation and thrombocytopenia in dengue hemorrhagic fever patients.

## MATERIALS AND METHODS

Patients admitted to the Pediatric Ward Dr Soetomo Hospital, from September 2000 to September 2001, with suspected dengue infections, according to the WHO criteria 1997 (Anonymous, 1997), were enrolled in the study. Written informed consent was obtained from children's parents or legal guardians. Blood specimens were obtained for complete blood count examination, determination of the plasma vWF level by Diagnostica Stago Asserachrom vWF Enzyme immunoassay (Boehringer Mannheim), detection of IgM and IgG using the immunochromato-

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graphic assay (Panbio, 2000). The vWF and platelet count were followed for three consecutive days starting from the first day of admission, to see the trend of relationship between the two items.

Descriptive analysis was used to show the characteristics of the patients, while correlation tests and multivariate analysis were used to see the trend of relationship between vWF and thrombocytopenia in DHF. Data were analyzed using SPSS for Windows, version 10.0.

## RESULTS

Between September 2000 and September 2001, 41 patients with clinical diagnosis of dengue virus infection were enrolled into the study. Ages ranged from 24 to 156 months, with an average of  $81.7317 \pm 36.5507$  months. The patients' temperatures ranged from  $36^\circ\text{--}40^\circ\text{C}$ , with an average of  $37.3829^\circ \pm 0.9711^\circ\text{C}$ . Patients were admitted after having suffered from fever for 2-6 days, with an average of  $4.4146 \pm 1.0718$  days. Most of the patients had normal weight (Fig 1). Eleven patients were serologically positive, showing both IgG and IgM anti-dengue antibodies, 19 were positive for IgG, and for the remaining 11 patients, who were serologically negative, the diagnosis was based on clinical grounds. Basic characteristics of the patients are listed in Table 1.

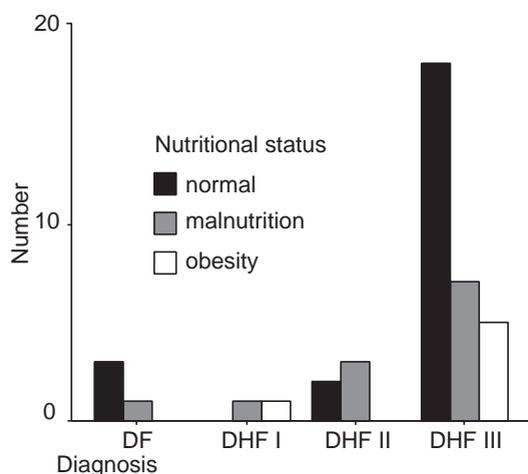


Fig 1—Diagnosis and nutritional status of children with dengue infections (N=41).

Correlations of vWF plasma levels and platelet counts are shown in Fig 2. The vWF showed a mean of  $174.2553 \pm 55.4891$  on the first day, on the second day the average vWF level was  $155.2395 \pm 50.5427$ , while on the third day the average was  $155.0211 \pm 55.4797$ . Sequential numbers of platelets were  $72,3947 \pm 39,8664$  on the first day,  $83,5263 \pm 53.0995$  and  $113,9737 \pm 59,8378$  on the second and third days, respectively.

Figs 2 and 3 are reflections of correlations between changes in vWF levels and platelet counts. The two figures are very much alike, which could have been due to the fact that DHF grade III accounted for almost three fourths of the population samples. Daily observation showed a significant change in vWF levels ( $p = 0.000$ ).

Table 1  
Characteristics of 41 patients with dengue infections.

Patient's characteristic	Number of patients (%)
Age (months)	$81.7317 \pm 36.5507$
Days since onset of disease	$4.4146 \pm 1.0718$
Sex	
Male	19 (46.3%)
Female	22 (53.7%)
Clinical diagnosis	
Dengue fever	4 (9.8%)
DHF grade I	2 (4.9%)
DHF grade II	5 (12.2%)
DHF grade III	30 (73.2%)
Nutritional status	
Normal	23 (56.1%)
Malnutrition	12 (29.3%)
Obesity	6 (14.6%)
Temperature ( $^\circ\text{C}$ )	$37.3829 \pm 0.9711$
Bleeding manifestations	
Positive tourniquet test	19 (46.3%)
Skin bleeding (petechiae, purpura, hematoma)	19 (46.3%)
Epistaxis	12 (29.3%)
Hematemesis	1 (2.4%)
Melena	2 (4.9%)
Serologic evidence	
IgG and IgM positive	11 (26.8%)
IgG positive	19 (46.3%)
Negative	11 (26.8%)

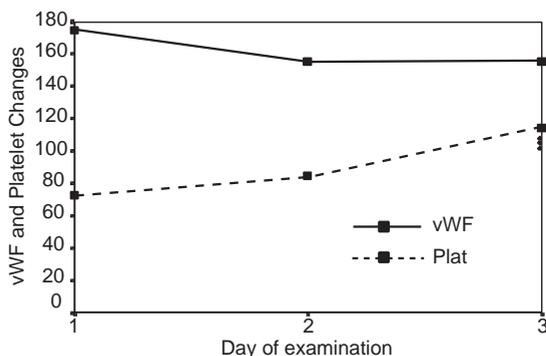


Fig 2—von Willebrand factor and platelet changes of patients with dengue infection in three consecutive days.

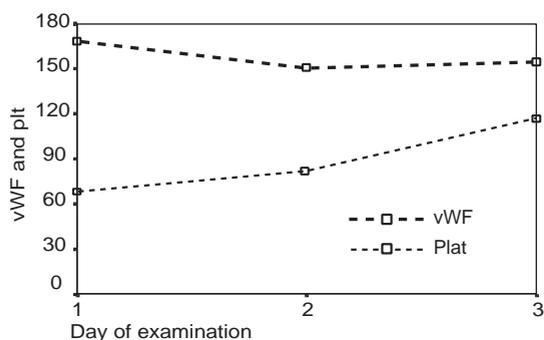


Fig 3—Laboratory results for vWF and platelet counts in DHF III.

The platelet count, which was obviously low on the first day of observation, steadily increased from day to day reaching a significantly higher level on the third day ( $p = 0.002$ ). The multivariate test for correlations between vWF and platelet count revealed no significant correlation ( $p = 0.988$ ).

By doing the Pearson correlation, vWF did not correlate with evidence of plasma leakage, where the significance yielded  $p = 0.216$ , nor did it with signs of bleeding as shown by the low significance for epistaxis ( $p = 0.360, 0.382$ , and  $0.525$  along with  $r = 0.147, 0.142$ , and  $0.106$  on examination-days 1, 2, and 3, respectively), petechiae (sequential  $p$  of  $0.711, 0.686$ , and  $0.675$ , and  $r = 0.060, 0.066$ , and  $0.070$ ), hematemesis ( $p = 0.935, 0.666$ , and  $0.447$  with  $r = -0.13, -0.70$ , and  $0.127$  on three consecutive days), and melena ( $p = 0.804, 0.464$ , and  $0.684$ , and  $r = -0.40, 0.119$ , and  $0.684$ ).

## DISCUSSION

von Willebrand factor (vWF) is a high molecular weight glycoprotein, which is synthesized by endothelial cells and megakaryocytes (Schröter *et al*, 1987). It is present within endothelial cells, the sub-endothelial matrix, in plasma and in platelets (Pannekoek and Voorberg, 1989). The endothelial source appears to be the major contributor to plasma vWF, which circulates in plasma at concentrations of  $10 \mu\text{g/ml}$ . Within endothelial cells, vWF has been identified in the cytoplasm, on the plasma membrane and particularly in the endoplasmic reticulum and in specific cytoplasmic organelles, the Weibel-Palade bodies (Booth *et al*, 1987). The release of functional vWF from endothelial cells exposed to inflammatory mediators may be a mechanism for localizing platelets and enhanced thrombogenicity at inflammatory foci (Schröter *et al*, 1987). Synthesis in the megakaryocytic cell type is responsible for the occurrence of vWF in the  $\alpha$ -granules of platelets. The activation of platelets with substances such as ADP causes the release of vWF in the plasma milieu. There is good evidence that the endothelium is the major source of plasma vWF. Von Willebrand factor is also acutely released *in vivo* in response to a variety of biological mediators, notably vasopressin, and from endothelial cells *in vitro* in response to thrombin (Pottinger *et al*, 1989). The principal functional roles for vWF appear to be in platelet adhesion to the vessel wall and as a carrier for coagulation factor VIII in the plasma. In hemostasis, vWF functions to support platelet plug formation at sites of vascular injury, by binding to the exposed subendothelium, forming a bridge between this surface and platelets (platelet-surface interactions). These functions are facilitated by the peculiar structure of vWF, arranged in multimers, and by the presence or exposure on the platelet membrane of glycoproteins that function as receptors for vWF, the glycoprotein Ib, and the glycoprotein complex Ib/IIIa (Mannucci, 1995).

At least two circumstances lead to increases in circulating vWF Ag; one is associated with acute inflammatory reactions and produces a transient elevation during the acute phase response, the other produces long-term rises in vWF Ag in

disease with vascular involvement. Significant changes in endothelial cell functions regulating hemostasis and thrombosis have been demonstrated following exposure to cytokines such as interleukin-1 and tumor necrosis factor or bacterial lipopolysaccharide, which itself induces endothelial interleukin-1 production. It is, therefore, plausible to suggest that acute elevations of vWF Ag are due to the interaction of polysaccharides or cytokines such as interleukin-1 with endothelium. (Pottinger *et al*, 1989).

Dengue hemorrhagic fever is primarily a disease of children below 15 years, although it may also occur in adults. Prior immunity to a serotype of dengue infection is a feature of more than 90% of cases, resulting in immune enhancement and massive release of cytokines with vasoactive properties (Gubler, 1998; van Gorp *et al*, 1999; Franchini *et al*, 2000; Halstead, 2000). These vasoactive mediators cause capillary leakage, circulatory failure and disseminated intravascular coagulopathy. The mechanism of bleeding in DHF is not known, but mild degrees of disseminated intravascular coagulation, liver damage, and thrombocytopenia may operate synergistically (Leangpibul and Thongcharoen, 1993). Mechanisms of hemorrhage that may be involved in DHF, as proposed by other authors, include thrombocytopenia, platelet dysfunction, reduced levels of coagulation factors, disseminated intravascular coagulation (DIC) and vascular (endothelial cell) injury (Franchini *et al*, 2000). Skin hemorrhages are the most common, including petechiae and purpura, as well as gum bleeding, epistaxis, menorrhagia and gastrointestinal hemorrhage. A blood test will usually show thrombocytopenia (platelet count  $\leq$  100,000/ml) and evidence of vascular leak syndrome (hemoconcentration) (Halstead, 2000). Signs of bleeding were also seen in this study, along with thrombocytopenia and elevated levels of vWF, although significant correlations were not evident. This could have been due to the fact that, although mechanisms of bleeding have not been well defined, thrombocytopenia and vWF were not the only contributors.

Fig 2 shows that although the high level of a vWF obviously decreased on the second observation day, it did not prove to do so the following

day, showing an almost flat change in the vWF level. However, it would be too early to conclude from a three-day observation, since acute infectious illnesses may lead to a significant elevation of plasma vWF concentrations, >3-fold above normal, which is expected to return to normal levels within 3-4 weeks (Pottinger *et al*, 1989). Multivariate tests resulted in a significant decrease in plasma vWF levels ( $p = 0.000$ ).

In contrast to the vWF changes, the platelet count showed a steady increase on daily observation, and these platelet changes also proved to be significant ( $p = 0.002$ ) This was in accordance with previous findings, where the lowest platelet count would be encountered during the shock phase, to increase shortly thereafter in the convalescent phase, reaching normal value after sickness for seven to ten days (Gubler, 1998; Halstead, 2000).

Multivariate analysis did not show significant correlations between vWF and platelet count when plotted to patients with DHF ( $p = 0.988$ ). From the limited time of observation, it is difficult to draw firm conclusions relating vWF and thrombocytopenia in DHF, and one may speculate that by extending the length of observation a significant difference would be encountered. Besides, although it has been assumed that the vascular endothelium is one of the main targets for viruses to induce coagulation, where endothelial cells may turn into a procoagulant state either by stimulation of cytokines in concert with circulating blood cells, such as lymphocytes or platelets, or by direct infection of endothelial cells, bleeding in infectious disease is a multifactorial process, resulting from a combination of thrombocytopenia, consumption of clotting factors, (local) hyperfibrinolysis, and vascular damage or leakage. In addition, immunologically mediated vasculitis may contribute to bleeding (Rothman and Ennis, 1999).

#### ACKNOWLEDGEMENTS

The author thanks the following people for their contributions: Prof Soegeng Soegijanto, Department of Pediatrics, Dr Soetomo Hospital, Faculty of Medicine, Airlangga University, Surabaya; Dr Djoni Djunaedi, Department of Internal Medi-

cine, Faculty of Medicine, Brawijaya University, Malang; paramedical staff of the Infection and Tropical Pediatrics Ward, and Mrs Sandra from the Pediatric Laboratory, Dr Soetomo Hospital, Surabaya.

## REFERENCES

- Anonymous. Dengue haemorrhagic fever. Diagnosis, treatment, prevention and control. 2<sup>nd</sup> ed. Geneva: WHO, 1997.
- Booth F, Allington MJ, Cederholm-Williams SA. An *in vitro* model for the study of acute release of von Willebrand factor from human endothelial cells. *Br J Haematol* 1987; 67: 71-8.
- Franchini G, Ambinder RF, Barry M. Viral disease in hematology. *Hematology* (Am Soc Hematol Educ) 2000: 409-23.
- Gubler DJ. Dengue and dengue hemorrhagic fever. *Clin Microbiol Rev* 1998; 11: 480-96.
- Halstead SB. Dengue fever/dengue hemorrhagic fever. In: Behrman RE, Kliegman RM, Jenson HB, eds. Nelson textbook of pediatrics. 16<sup>th</sup> ed. Philadelphia: WB Saunders, 2000: 1005-7.
- Leangpibul P, Thongcharoen P. Clinical laboratory investigations. In: Thongcharoen P, compiler. Monograph on dengue/dengue hemorrhagic fever. New Delhi: World Health Organization. Regional Office for Southeast Asia. Regional Publication, SEARO. 1993; 22: 62-71.
- Mannucci PM. Platelet von Willebrand factor in inherited and acquired bleeding disorders. *Proc Natl Acad Sci USA* 1995; 92: 2428-32.
- Panbio. Panbio Dengue Duo IgM and IgG Rapid Strip Test (Leaflet). 2001.
- Pannekoek H, Voorberg J. Molecular cloning, expression and assembly of multimeric von Willebrand factor. *Bailliere's Clin Haematol* 1989; 2: 879-96.
- Pottinger BE, Read RC, Paleolog EM, Higgins PG, Pearson JD. von Willebrand factor is an acute phase reactant in man. *Thrombosis Res* 1989; 53: 387-94.
- Rigau-Perez JG, Clark GG, Gubler DJ, *et al.* Dengue and dengue hemorrhagic fever. *Lancet* 1998; 352: 971.
- Rothman AL, Ennis FA. Immunopathogenesis of dengue hemorrhagic fever. *Virology* 1999; 257: 1-6.
- Schrorer AE, Moldow CT, Rick ME. Interleukin 1 or endotoxin increases the release of von Willebrand factor from human endothelial cells. *Br J Haematol* 1987; 67: 193-7.
- van Gorp ECM, Minnema MC, Suharti C, *et al.* Activation of coagulation Factor XI, without detectable contact activation in dengue hemorrhagic fever. *Br J Haematol* 2001; 113: 94-9.
- van Gorp ECM, Suharti C, Setiati TE, *et al.* Coagulation disorders in children with viral hemorrhagic fever. *Clin Microbiol Infect* 1997; 3(suppl 2): 356.
- van Gorp ECM, Suharti C, ten Cate H, *et al.* Review: infectious diseases and coagulation disorders. *J Infect Dis* 1999; 180: 176-86.