Short Communication

Matrix Metalloproteinase-9 (MMP-9) in Children with Dengue Virus Infection

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(Received March 26, 2010. Accepted July 13, 2010)

SUMMARY: The purpose of this study was to investigate the role of matrix metalloproteinase-9 (MMP-9) in the pathogenesis of dengue vasculopathy in patients with dengue virus infection. Serum samples from 24 children with dengue were analyzed for MMP-9 during the febrile and toxic stages and at follow-up. Serum samples obtained from 7 healthy children were used as controls. Serum MMP-9 levels in patients with dengue virus infection were found to be lower at the febrile (227.0 ± 186.9 ng/ml) and toxic stages (150.9 ± 151.7 ng/ml) than at follow-up (424.5 ± 227.8 ng/ml) or in the control group (393.3 ± 125.9 ng/ml, P < 0.001 by one-way ANOVA). There was no significant difference between MMP-9 levels in patients with DHF and those with DF at any stage of the disease. In conclusion, MMP-9 levels are reduced during the febrile and toxic stages of dengue virus infection.

Dengue hemorrhagic fever (DHF) is one of the most important emerging infectious diseases in Thailand and worldwide (1,2). Plasma leakage, as evidenced by hemoconcentration, ascites, or pleural effusion, is the major pathophysiological hallmark that determines disease severity and distinguishes DHF from dengue fever (DF) (3). Matrix metalloproteinase-9 (MMP-9), which is an endopeptidase involved in degradation of the extracellular matrix, tissue remodeling, endothelial injury, and angiogenesis (4), has been demonstrated to be implicated in the development of several vascular conditions and diseases (5-7). However, its significance in patients with dengue virus infection has never been studied. We hypothesized that MMP-9 may play a crucial role in the mechanism of plasma leakage in DHF by causing injury to the extracellular matrix, thus resulting in endothelial cell swelling and detachment from the basement membrane. The objective of this study was to determine the level of MMP-9 in children with dengue virus infection.

Children hospitalized at the Had Yai Hospital, Songkhla, Thailand between January 2005 and December 2006, with an initial diagnosis of dengue virus infection were enrolled. Blood samples at the febrile and toxic stages, and at follow-up (at least 1 week after defervescence) were obtained from a total of 24 patients (age, 9.5 ± 2.4 years; male/female = 18/8; DF, 16; DHF, 8) and the serum MMP-9 level determined using a commercially available ELISA kit (QuantiKine R&D Systems, Minneapolis, Minn., USA). The dengue serotype was identified in 20 patients (7 each with DEN-1 and DEN-2 and 6 with DEN-4) using a polymerase chain reaction (PCR) method. The remaining patients had serologically confirmed dengue infection by enzyme-linked immunosorbent assay (ELISA). DHF diagnosis and grading was performed according to the criteria published by the World Health Organization (WHO) (3). The research protocol for this study was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University. Written informed consent was obtained from an appropriate guardian and/or the patient prior to enrollment.

MMP-9 levels were determined for a total of 61 serum samples. Seven healthy children served as normal controls. The demographic and clinical data for all subjects are summarized in Table 1. The serum MMP-9 levels in patients with dengue virus infection (DF + DHF) during different stages of the disease, and in controls, are shown in Figure 1. Serum MMP-9 was found to differ significantly between the three stages of the disease (P < 0.001 by one-way ANOVA). Post-hoc analysis showed that MMP-9 levels were significantly lower in patients with dengue virus infection at the febrile and

<table>
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<tr>
<th>Table 1. Demographic and clinical data of 24 patients enrolled</th>
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<tr>
<td>Age (yr)</td>
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<td>---------</td>
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<tr>
<td>9.4 ± 2.3</td>
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<tr>
<td>Sex (M/F)</td>
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<td>Body temperature (°C)</td>
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<td>Maximal hematocrit (%)</td>
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<td>Lowest platelet (×10^9/mm³)</td>
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DF: dengue fever; DHF: dengue hemorrhagic fever; NS, not significant; N/A, not applicable.

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toxic stages than at follow-up or in the control group (227.0 ± 186.9 and 150.9 ± 151.7 ng/ml for the febrile and toxic stages, respectively, versus 424.5 ± 227.8 and 393.3 ± 125.9 ng/ml for follow-up and the control group, respectively; P < 0.05 for febrile versus follow-up, toxic versus follow-up, and toxic versus control). Figure 2 shows the difference between MMP-9 levels in patients with DF and DHF at different stages of the disease. Although the serum MMP-9 level appears to be lower in DHF patients than in DF patients, the difference was not statistically significant.

Recent in vitro and mouse experiments have revealed the importance of MMPs in dengue virus infection-induced vascular leakage. Lupertlop et al. found that dengue virus-infected immature dendritic cells overproduced MMP-9 in a viral dose-dependent manner (8). The increase of this protein led to enhanced vascular permeability, which could be reduced by specific inhibitors. Moreover, the elevated level of tissue inhibitors of metalloproteinases (TIMP)-1, a natural inhibitor of MMP-9, was demonstrated (8). In another study, dengue virus infection of primary human endothelial cells strongly increased production of MMP-2 and, to a lesser extent, MMP-9 (9). Overproduction of MMP-9 was proposed to explain endothelial injury and vascular leakage in children with DHF.

Contrary to the original hypothesis, our study has demonstrated that MMP-9 levels are not elevated during the febrile or toxic stages of dengue virus infection. Furthermore, our findings do not support the role of MMP-9 in the pathogenesis of dengue virus-related illness. The reason for the lower MMP-9 levels in patients with dengue virus infection at the toxic stage, especially in DHF, is unclear. One possible reason to explain this finding is that the level of MMP inhibitors, such as TIMPs and α2-macroglobulin, in the serum of dengue-infected patients may increase rapidly in an attempt to restore normal physiological function, as shown in an in vitro study. Further studies to explain the cause of lower MMP-9 levels in vivo, and the potential role of its inhibitors in the pathophysiological progress of dengue virus infection, are therefore warranted.

**Acknowledgments** We would like to express our deep gratitude to the Commission on Higher Education, Ministry of Education, Department of Medical Sciences, Ministry of Public Health, and Center of Excellence in Viral Hepatitis, Thailand Research Fund, Center of Excellence in Clinical Virology, Chulalongkorn University, CU Centenary Academic Development Project and King Chulalongkorn Memorial Hospital for their generous support.

The study was funded by the Thailand Research Fund and Commission on Higher Education (Dr. Apichai Khongphatthanayothon, Grant number RMU4900019).

**REFERENCES**

3. World Health Organization (1997): Dengue haemorrhagic fever:

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**Fig. 1.** MMP-9 level in children with dengue infection during different stages of the illness (P < 0.001 by one-way analysis of variance). FU, follow-up.

**Fig. 2.** MMP-9 levels in patients with dengue fever (DF) versus dengue hemorrhagic fever (DHF) at different stages of the illness. NS, not significant.
diagnosis, treatments, prevention and control. 2nd ed. World Health Organization, Geneva.