CORRELATION OF SERUM LEVELS OF MACROPHAGE MIGRATION INHIBITORY FACTOR WITH DISEASE SEVERITY AND CLINICAL OUTCOME IN DENGUE PATIENTS

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Abstract. Dengue virus infection can cause mild dengue fever (DF) or severe dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Cytokines are believed to be involved in the pathogenesis of dengue infection. However, the role of the pro-inflammatory cytokine macrophage migration inhibitory factor (MIF) in dengue infection is unclear. In this study, serum levels of MIF in adult dengue patients with different disease severity and clinical outcome were determined and compared with the levels of other cytokines, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-10, and interferon gamma (IFN-γ), in the same patients. Serum levels of MIF, IL-6, and IL-10, but not IFN-γ or TNF-α, were higher in all DHF patients who died than in DHF survivors and DF patients. We conclude that in addition to IL-6 and IL-10, elevated levels of serum MIF are a potential predictor of disease severity and clinical outcome in dengue patients.

INTRODUCTION

Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine that plays an important role in the modulation of inflammatory and immune responses.1 This factor was originally described as a T lymphocyte protein that inhibited the random migration of macrophages. More recently, however, we have learned that MIF is a hormone released by different cells in many tissues in response to a variety of stimuli.2 Once released, MIF augments the secretion of tumor necrosis factor-α (TNF-α) and counteracts the anti-inflammatory action of glucocorticoids.3 Serum levels of MIF are increased in patients with systemic inflammatory response syndrome, sepsis, septic shock, and trauma,4-7 and correlate with death caused by sepsis.8 In addition, neutralizing antibodies against MIF result in better survival of mice with lethal septic shock after peritonitis infection with Escherichia coli.9

Dengue viruses are mosquito-borne flaviviruses subgrouped into four antigenically related serotypes: types 1, 2, 3, and 4.10 It is estimated that more than 50 million infection with dengue virus occur globally each year.11 Infection with dengue virus generally causes mild symptoms such as fever, headache, and muscle and joint pain, which is called dengue fever (DF). In some cases, however, infection with dengue virus may progress to dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). Dengue hemorrhagic fever is a severe febrile disease characterized by abnormalities in homeostasis and increased capillary leakage that can progress to hypovolemic shock (DSS). According to the criteria of the World Health Organization (WHO), four grades of DHF/DSS are recognized from the least severe grade I to the most severe grade IV. Grades III and IV are also referred to as DSS.12

Because the process leading to DHF/DSS is not fully understood, only supportive treatment is available, and DHF/DSS can result in high mortality rates.13 Many reports indicate that serum levels of proinflammatory cytokines such as TNF-α and interleukin-6 (IL-6), as well as Th1 (interferon-γ [IFN-γ]) and Th2 (IL-10) cytokines, are significantly increased in DHF patients. Most of these studies, however, are focused on children. In addition, it is unclear whether MIF is involved in the pathogenesis of DHF/DSS.

Therefore, in this study, we compared the serum levels of MIF, IL-6, TNF-α, IFN-γ, and IL-10 in adult patients with different disease severities of dengue infection. Our results demonstrated that the serum levels of MIF, as well as those of IL-6 and IL-10, were significantly increased in adult dengue patients, and that the serum levels of MIF correlated with disease severity and death in adult DHF patients.

MATERIALS AND METHODS

Dengue patients and serum samples. This study was performed with the approval of the Ethics Committee of National Cheng Kung University Hospital and with the consent of the participating patients. Sera were obtained from 32 dengue patients in the acute stage of disease between 1 and 18 days after fever onset (Table 1) during an outbreak of infection with dengue virus type 2 between August and October 2002 in southern Taiwan.12 Twelve cases were classified as DF and 20 were classified as DHF according to the criteria of the WHO.12 All 32 patients had high fever, headache, and muscle and joint pain, but only DHF patients were hospitalized for treatment. The diagnosis was further confirmed using an enzyme-linked immunosorbent assay (ELISA) for IgM and IgG antibodies to dengue virus or hemagglutination inhibition with or without virus isolation by the National Quarantine Service of the Taiwan Department of Health. A positive serologic result was defined as a ≥ 4-fold change in reciprocal IgG antibody titers to one or more dengue virus antigens in paired serum samples or a positive IgM antibody ELISA result on late acute-phase or convalescent-phase serum specimens. Additional serum samples of healthy adults (n = 17) without antibodies against dengue virus obtained during routine health examinations in this study were used as controls. All sera were collected and stored at −80°C until used.
Cytokine measurements. The MIF levels in the double-blind sera samples were measured using a commercially available ELISA kit (R & D Systems, Minneapolis, MN). Briefly, a monoclonal antibody to MIF (concentration = 200 ng/mL) was used as a capture antibody in combination with biotinylated MIF affinity-purified polyclonal detection antibody (concentration = 2.0 μg/mL). A standard curve was generated using a two-fold serial dilution of recombinant human MIF between 2 ng/mL and 30 pg/mL. Serum IL-6, TNF-α, IFN-γ, and IL-10 levels were also measured using ELISA kits (R & D Systems) according to the manufacturer’s instructions.

Statistical analysis. Data are expressed as the mean ± SE. Differences between the test and control groups were analyzed using the Mann-Whitney test. Significance was set at \( P < 0.05 \). Receiver operating characteristic (ROC) curves, which were made by plotting the true-positive rate (sensitivity) versus the false-positive rate (1 – specificity), were used to evaluate the diagnostic performance of inflammatory mediators at various cutoff points. An area under the ROC curve (AUROC) closer to 1 indicates greater discriminatory power, whereas an AUROC of 0.5 indicates no diagnostic potential. The optimum cutoff was calculated as the maximum value of sensitivity multiplied by specificity. Using standard formulas, we calculated sensitivity, specificity, and positive and negative predictive values for the cutoff that represented the best discrimination derived from the ROC curves.

RESULTS

Clinical characteristics and cytokine profiles of dengue patients. Of the 20 DHF patients included in this study, 7 patients died (non-survivors). Three of the seven non-survivors had diabetes mellitus (Table 1); however, none had immunosuppression or concomitant bacterial sepsis. The results of the ELISA for IgM and IgG antibodies to dengue virus showed that 17 (85%) of the 20 DHF patients had a secondary dengue infection, but only 2 (17%) of 12 DF patients had a secondary dengue infection (Table 1). No significant difference was found in the study day of serum collected after fever onset in DHF non-survivors (5.7 ± 1.5 days), DHF survivors (7.9 ± 1.7 days), and DF patients (5.1 ± 0.5 days) (Table 1). There were also no significant differences between the age and sex of dengue patients and controls (Table 2). However, significantly larger increases in MIF, IL-6, and IL-10, but not TNF-α, were found in DHF non-survivors than in survivors. Conversely, IFN-γ decreased more in DHF non-survivors than in survivors. All DHF non-survivors showed significantly larger increases in MIF, IL-6, and IL-10, but not TNF-α, than did DF patients (Table 2 and Figure 1). However, DHF survivors showed significantly larger increases only in serum levels of MIF than did DF patients. Serum levels of MIF, IL-6, and IFN-γ, but not IL-10 or TNF-α, were also more significantly increased in DF patients than in healthy controls (Table 2 and Figure 1).
Levels of MIF, IL-6, and IL-10 as predictive markers of death for DHF patients. The AUROC were calculated to analyze the discriminatory power of MIF, IL-6, and IL-10 levels for the prediction of fatal outcome of DHF patients. The AUROC was highest for MIF levels (AUROC = 0.967; 95% confidence interval [CI] = 0.89–1.04), followed by IL-6 levels (AUROC = 0.912; 95% CI = 0.78–1.04) and IL-10 levels (AUROC = 0.857; 95% CI = 0.65–1.06), indicating that MIF has the greatest discriminatory power as the predictor of fatal outcome. The optimum cutoff for MIF, which was determined using an ROC curve, was 54.7 ng/mL. At this cutoff, MIF had a sensitivity of 100% in identifying dengue patients who would die of the disease. The sensitivity, specificity, and positive and negative predictive values of MIF, IL-6, and IL-10 in DHF patients are shown in Table 3. Although the positive predictive value of MIF levels was higher than that of IL-6 levels (100% versus 77.8%), the negative predictive value of IL-6 was higher than that of MIF (100% versus 92.9%). The positive and negative predictive values of IL-10 were 83.3% and 85.7%, respectively, which suggests that MIF and IL-6 are the important markers for both types of prediction.

**DISCUSSION**

In this study, we demonstrated that in addition to IL-6 and IL-10, serum levels of MIF were also higher in adult DHF non-survivors than in DHF survivors and DF patients. Unlike the predominant pediatric patients with DHF/DSS in southeast Asia, all DHF patients in this study were adults. Three of the 7 DHF non-survivors had diabetes mellitus, a known cause of macrophage dysfunction. This indicates diabetes can be a risk factor for mortality in adult DHF patients. A subgroup analysis of these patients compared with the other non-survivors, especially for the macrophage-related cytokines, should be done.

Different hypotheses, including antibody-dependent enhancement, have been proposed to explain the mechanism of DHF/DSS, but this has yet to be fully elucidated. Early immune activation and cytokine production are related to the development of plasma leakage and disease severity in DHF/DSS. Pre-existing heterotypic antibodies to dengue may augment the infection of monocytes or macrophages by dengue virus. More dengue virus-infected monocytes may result in T cell activation; the activation of both types of cells may result in the elevation of proinflammatory cytokines, as well as Th1 and Th2 cytokine production. Up-regulation of these cytokines may contribute to the increase of vascular permeability, as well as to the initiation of coagulation and fibrinolysis during DHF/DSS. Our data are consistent with those of studies suggesting that overproduction of IL-6 and IL-10 is important in the pathogenesis of dengue virus infection.

We also found that IFN-γ was lower in DHF nonsurvivors than in survivors. A decrease in IFN-γ production in DHF nonsurvivors may reflect the loss of IFN-γ in activating cytotoxic T cells to clear dengue virus infection in these patients. These results suggest that higher IFN-γ levels in DHF patients may enable them to survive. Thus, DHF patients who are unable to mount a Th1 response and IFN-γ production may be more likely to have a fatal outcome.

We did not find a significant increase of serum levels of TNF-α in our dengue patients. This discrepancy may have been caused by the transience of TNF-α production; it is possible that we missed the peak of TNF-α production in our patients when we collected serum samples. Therefore, a kinetic study is required to further understand the dynamic changes of these cytokines during dengue virus infection.

In addition to IL-6 and IL-10, increased serum MIF levels in DHF patients correlated with a poor prognosis. Interestingly, serum levels of MIF were more useful in predicting death than were IL-6 levels (AUROC = 0.967 versus 0.912). The positive predictive value of MIF levels was also higher than that of IL-6 (100% versus 77.8%). In addition, only MIF was significantly increased in DHF survivors than in DF patients. Our report is the first to demonstrate that serum levels of MIF correlate with disease severity in dengue infection. However, due to the small population of adult dengue patients in the present study, further studies with larger populations of dengue patients that include infants and children...
are necessary to verify that this finding is true for all DHF patients. The mechanism that induces MIF production during dengue infection is still unclear, but it is possible that both virus infection and thrombin stimulation may be the triggers.  

Our results suggest that the uncontrolled overproduction of MIF found in bacterial sepsis and systemic inflammatory response syndrome may also be involved in the pathogenesis of DHF/DSS.

In summary, we demonstrated that in adult dengue patients, serum levels of MIF, IL-6, and IL-10 were increased...
TABLE 3

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<thead>
<tr>
<th></th>
<th>MIF (μg/mL)</th>
<th>IL-6 (pg/mL)</th>
<th>IL-10 (pg/mL)</th>
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<tr>
<td>Cutoff value</td>
<td>54.7</td>
<td>68.6</td>
<td>267.8</td>
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<td>Sensitivity (%)</td>
<td>85.7</td>
<td>100.0</td>
<td>71.4</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>100.0</td>
<td>84.6</td>
<td>92.3</td>
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| Positive predictive value (%) | 100.0 | 77.8 | 83.3 |
| Negative predictive value (%) | 92.9  | 100.0| 85.7 |

AUROC ± SE 0.967 ± 0.037 0.912 ± 0.067 0.857 ± 0.104

95% CI 0.89–1.04 0.78–1.04 0.65–1.06

* MIF = macrophage migration inhibitory factor; IL-6 = interleukin-6; DHF = dengue hemorrhagic fever; AUROC = area under receiver operating characteristic curve; CI = confidence interval.

and positively correlated with disease severity and fatal outcome in DHF patients. In addition, considering the high sensitivity of serum IL-6 levels and the high specificity of MIF levels in the prediction of mortality in DHF patients, these two cytokines should be monitored in dengue patients to let clinicians know which patients need treatment most urgently.

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