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# The role of macrophage Migration Inhibitory Factor (MIF) in pediatric dengue infection at Sanglah Hospital, Bali, Indonesia



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## ABSTRACT

**Background:** Macrophage migration inhibitory factor (MIF) is known had pleiotropic properties particularly participating in inflammatory and immune responses. MIF has initially involved phagocytosis, spreading, and tumoricidal activity in macrophage. Recently, MIF was determined as a proinflammatory cytokine that has a pivotal role in viral infection such as dengue. This study aims to evaluate the role of MIF levels in patients with dengue infection at Sanglah General Hospital, Bali, Indonesia

**Methods:** A cross-sectional study was conducted among 48 children aged 1 to 12 years who hospitalized in the Children's Ward of Sanglah General Hospital, Bali, Indonesia, from August 2016 to July 2017. The respondents were divided into two groups: 24 children with a diagnosis of Dengue Shock Syndrome (DSS) (Group 1) and 24 non-DSS children. Data regarding age group, gender, obesity status, history of secondary infection, grading for dengue infection, and levels of MIF were assessed in this study. The MIF levels between both groups were evaluated and analyzed using SPSS version 23 for Mac.

**Results:** Most of the patients were age > 5 years old in Non-DSS (87.5%) and DSS (54.2%) groups. However, females were predominant in the Non-DSS group (66.7%), but males in the DSS group (58.3%). The non-obesity history was more frequent in both Non-DSS (87.5%) and DSS (87.5%) groups. But, the history of secondary infection was more common in the DSS (70.8%) compared with a non-DSS group (37.5%). A significant difference in mean MIF levels was found between patients with DSS (102 (42.91-141.12) ng/ml) and non-DSS (24.85 (12.61-50.80) ng/ml) ( $p < 0.001$ ). MIF levels were significantly different between several degrees of dengue infection ( $P < 0.05$ ).

**Conclusions:** Serum MIF levels in non-DSS patients significantly differ from MIF levels in DSS patients. MIF serum levels increase in accordance with the increasing degree of severity of dengue infection. This data shows that MIF has a role in the occurrence of severe dengue infection.

**Keywords:** MIF, Dengue Infection, Macrophage, Pediatric

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## INTRODUCTION

Many factors play a role in the onset of severe dengue infection. One of the cytokines that play a role in infection is macrophage *Migration Inhibitory Factor* (MIF).<sup>1</sup> Macrophage MIF has the ability to stimulate monocytes to secrete pro-inflammatory cytokines or other mediators, such as *Tumor Necrosis Factor- $\alpha$*  (TNF- $\alpha$ ), *Interleukin 1 $\beta$*  (IL-1 $\beta$ ), and *matrix metalloproteinase* (MMP).<sup>1</sup> The effect of increasing various cytokines will induce plasma leakage and cause coagulation disorders.<sup>2</sup> The occurrence of plasma leakage is also exacerbated by damage to the *Zonula-Occcludens 1* (ZO-1) protein at the *tight junction* induced by MIF.<sup>3</sup> This mechanism will increase the risk of shock in dengue infection.<sup>1,3</sup>

Research conducted in vitro shows that MIF plays a role in many ways in the pathogenesis of dengue infection including increasing viral replication, increasing vascular permeability and affecting the coagulation system.<sup>1-3</sup> In vitro study was conducted on *Human Umbilical Cord Vein* (HUVEC) media showed that dengue virus

infection would induce expression of MIF.<sup>4</sup> This expression will stimulate the production of thrombomodulin and *Intercellular Adhesion Molecule* (ICAM).<sup>4</sup> All of the above mechanisms are closely related to the occurrence of plasma leakage and coagulation disorders.

Several studies in humans have been conducted to prove the relationship between MIF and the severity of dengue infection. A previous study conducted on adult subjects by Chen LC et al. showed that mean MIF levels were significantly higher in living *Dengue Hemorrhagic Fever* (DHF) patients compared to *Dengue Fever* (DF) patients.<sup>5</sup> MIF levels were also found to be higher and significantly different in DHF patients who died than those who lived.<sup>5</sup> The results showed that MIF has a specificity (100%) better in predicting mortality than *interleukin 6* (IL-6) and *interleukin 10* (IL-10).<sup>5</sup>

Another study conducted by Malavige GN et al., in 2013 found that the 259 adult patients suggest controversial results.<sup>6</sup> Serum MIF levels were found to be higher in severe dengue infections than

non-severe dengue infections, but this difference was not statistically significant.<sup>6</sup>

Macrophage MIF has a critical role that causes the clinical outcome of dengue infection to become more severe, but research on MIF in children is still very limited. Research on children was carried out by Ferreira RA et al., in 2015, among 138 children in Brazil.<sup>7</sup> The results of this study indicate that MIF does not have a significant relationship with other pro-inflammatory cytokines such as *Interferon-γ* (IFN  $\gamma$ ), IL 10, IL 13, and TNF  $\alpha$ .<sup>7</sup> However, the relationship between MIF and the clinical degree of dengue infection was not investigated in this study.

Based on those mentioned above, this study aims to determine the relationship between levels of MIF with the incidence of severe dengue and determine levels of MIF in children with various degrees of dengue infection.

## METHOD

A cross-sectional analytic study was conducted to determine the relationship of serum MIF levels with *dengue shock syndrome* (DSS) and to know levels of MIF in various degrees of dengue infection of children. The study was conducted at the Department of Child Health/ Sanglah Hospital Denpasar. The study was conducted in August 2016-July 2017.

The affordable population is all children aged between 1 year to 12 years with suspected dengue infection, who are hospitalized in the Department of Child Health Sanglah Hospital Denpasar from August 2016 to July 2017. Subjects were determined through consecutive sampling in which subjects were collected by sequentially during the study period.

Inclusion criteria are children who have fever day 5 to day 6 with platelet levels of  $\leq 100,000$  cells / mm<sup>3</sup> and leukocytes  $\leq 5000$  cells/mm<sup>3</sup>. The exclusion criteria are children suffering from sepsis, with autoimmune diseases, children with *Human Immunodeficiency Virus* (HIV), suffering from malignancies and metabolic diseases such as diabetes mellitus (DM), using steroid drugs, and children who are proven not to be dengue infection by serological examination.

The sample size is calculated by calculating the sample size to test the hypothesis of the mean difference of 2 independent populations with a type I error of 5% one-way hypothesis, a type II error of 20%. From the literature, it is known that the mean MIF level in DHF is 47.2 ng/ml  $\pm$  14.3 ng/ml and the mean MIF control level is 33.1 ng/ml  $\pm$  14.0 ng/ml.<sup>4</sup> The difference in MIF that was considered significant was 10 ng/ml, the minimum sample size of each group required was 24 samples.

Children who took care at Sanglah General Hospital with suspected dengue infection that met the inclusion and exclusion criteria were made as research subjects after informed concern was conducted. Materials used in the study were blood samples taken from subjects using syringes taken as much as  $\pm 5$  ml of blood for the examination of MIF levels and as much as  $\pm 3$  ml of blood for anti-dengue serological examination.

The MIF level measuring device, namely a micro-plate reader, checks are carried out in the Prodia® Denpasar laboratory. The required sample is 5 ml of blood in a vacutainer containing EDTA, heparin or citrate as an anticoagulant. This sample will then be centrifuged and stored at  $<-20^{\circ}$  C. MIF examination is carried out if the subject is confirmed to be suffering from dengue infection (eligible subject) as seen from the *non-structural protein 1* (NS1) or anti-dengue serology. Anti-dengue serology examination using Panbio Pty Ltd® dengue rapid strip test. Anti-dengue serological examination is carried out during the 6th or 7th day of fever and examined in the clinical pathology laboratory of Sanglah Hospital.

The descriptive analysis illustrates the characteristics of the subjects between shock groups and not shock groups. The Mann-Whitney test was performed on DSS and non-DSS data. Kruskal Wallis and Mann-Whitney post hoc tests were performed to see the relationship of MIF levels with each degree of dengue. The relationships were considered statistically significant if the p-value is less than 0.05. Data were analyzed using SPSS version 23 for Mac.

## RESULTS

Most of the respondents in Non-DSS group were age  $> 5$  years old (87.5%) similar to the DSS group (54.2%) (Table 1). Based on gender, the female was predominant in Non-DSS group (66.7%) compared with the male in the DSS group (58.3%). There is no history of obesity between both Non-DSS (87.5%) and DSS (87.5%) groups (Table 1). However, based on the history of secondary infection, DSS group were more frequent (70.8%) compared with the Non-DSS group (37.5%) (Table 1).

Most of the patients were diagnosed by DHF grade IV (33.33%), followed by Dengue Fever (31.25%), DHF grade III (16.67%), DHF grade I (14.58%), and DHF grade II (4.17%) (Table 2). There was a significant different in the median value of MIF levels ( $p < 0.001$ ) between DHF grade IV with 121.39 (62.30-141.12) ng/ml, followed by 57.72 (42.91-139.44) ng/ml in DHF grade III, 40.30 (29.80-50.80) ng/ml in DHF

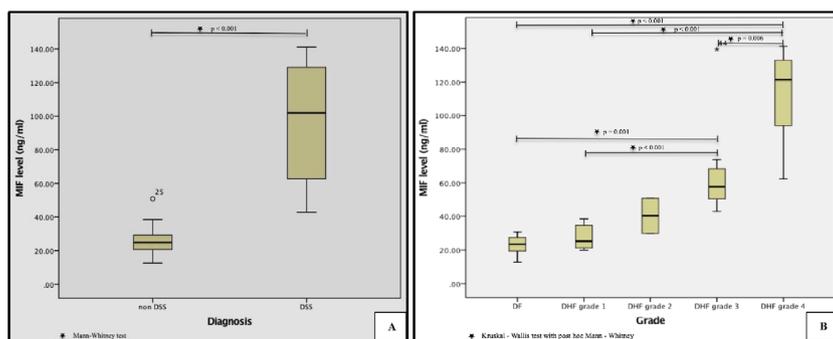
**Table 1** Baseline characteristic of respondents

Characteristics	Dengue Infection (N=48)	
	Non-DSS (n=24)	DSS (n=24)
<b>Age group (year), n (%)</b>		
≤ 5 year	3 (12.5)	11 (45.8)
> 5 year	21 (87.5)	13 (54.2)
<b>Gender, n (%)</b>		
Female	16 (66.7)	10 (41.7)
Male	8 (33.3)	14 (58.3)
<b>Obesity, n (%)</b>		
Yes	3 (12.5)	3 (12.5)
No	21 (87.5)	21 (87.5)
<b>Secondary infection, n (%)</b>		
Yes	9 (37.5)	17 (70.8)
No	15 (62.5)	7 (29.2)

**Table 2** The levels of MIF levels according to grade and between groups

Variables	N (%)	Median value of MIF levels (maximum-minimum)	p
<b>Dengue Grade (ng/ml)</b>			
Dengue Fever	15 (31.25)	23.28 (12.61-30.70)	< 0.001
DHF grade I	7 (14.58)	25.10 (19.90-38.50)	
DHF grade II	2 (4.17)	40.30 (29.80-50.80)	
DHF grade III	8 (16.67)	57.72 (42.91-139.44)	
DHF grade IV	16 (33.33)	121.39 (62.30-141.12)	
<b>MIF levels (ng/ml)</b>			
Non-DSS	24 (50.00)	24.85 (12.61-50.80)	0.001
DSS	24 (50.00)	102.00 (42.91-141.12)	

Kruskal-Wallis test; Post hoc Mann-Whitney: DF vs DHF grade IV p < 0.001; DHF grade I vs DHF grade III p < 0.001; DHF grade I vs DHF grade IV p < 0.001; DHF grade III vs DHF grade IV p = 0.006; p-value less than 0.05 was considered statistically significant.



**Figure 1** (A) MIF Levels in DSS and non-DSS as well as (B) in various degrees of dengue infection

grade II, 25.10 (19.90-38.50 ng/ml in DHF grade I, and 23.28 (12.61-30.70) ng/ml in Dengue Fever (Table 2). The MIF levels between groups also suggest a significantly different (p=0.001) in DSS

(102.00 (42.91-141.12) ng/ml) and Non-DSS (24.85 (12.61-50.80)) group using Kruskal-Wallis test (Table 2 and Figure 1). The Post-Hoc Mann-Whitney test was also confirmed the statistically significantly different between a group of Dengue Grade (P<0.05) (Table 2 and Figure 1).

**DISCUSSION**

This study is conducting the relationship of MIF levels only in cases of severe dengue infection and non-severe dengue infection. This study also compared MIF levels among each grade in dengue infection. The influence of other factors besides MIF levels for the occurrence of severe dengue is very much, but in this study were not analyzed (report in the other publication).

The data shows the significant value of MIF levels that are significant between cases of severe dengue (DSS) and those that are not severe (non-DSS). This data illustrates that MIF has a considerable role to play in cases of severe dengue. A previous study also obtained similar results such as conducted by Yeh TM et al., in 2013 where the infection by the DEN2 virus on HUVEC media increases MIF expression.<sup>4</sup> Increased MIF expression plays a role in the occurrence of coagulation disorders that aggravate the degree of dengue infection.<sup>8</sup>

The mechanism of dengue virus induces MIF secretion is still unclear. In vitro research shows that the DEN2 virus will activate NF-κB through phosphorylation and degradation of protein inhibitors (IκB).<sup>9</sup> Activation will cause NF-κB to be separated from plasma and translocation into the nucleus to begin the process of transcription of MIF genes.<sup>9</sup> Another in-vitro study also showed the role of NS1 in MIF production.<sup>10</sup> The administration of recombinant NS1 in *Human Endothelial Cell Line 1* (HMEC-1) media has been shown to increase MIF production.<sup>10</sup>

Macrophage MIF will induce *T-helper type 1* (Th1) cells to produce several cytokines such as IFN γ, IL-12 and TNF α that will cause the autophagy process.<sup>11</sup> With this autophagy process, the dengue virus is not killed but can replicate in the cells where the autophagy process occurred. Increased levels of MIF will cause overproduction of other pro-inflammatory cytokines thereby increasing the risk of more severe infections.<sup>11</sup>

The mechanism of MIF in causing plasma leakage is not yet fully understood. Some in vitro studies finding MIF can induce several inflammatory mediators that play a role in causing plasma leakage.<sup>1-3</sup> These mediators include TNF-α, *matrix metalloproteinase* (MMP), IL-1β, and *vascular endothelial growth factor* (VEGF).<sup>1</sup> Study in-vitro

shows that MIF can directly induce plasma leakage through damage to one of the junction proteins such as zonula occluden-1 (ZO-1).<sup>12</sup> Activation occurs through MIF binding with *Chemokine Receptor Type-4* (CXCR4) and *Cluster of Differentiation 74* (CD74) on the surface of endothelial cells.<sup>13,14</sup> This mechanism will cause the tight junction to loosen, plasma leakage occurs resulting in shock.<sup>1,15</sup>

Study in-vitro by Chuang YC in 2011 showed that dengue virus infection on HMEC-1 media was able to induce MIF expression resulting in increased production of TNF  $\alpha$ , *matrix metalloproteinase* (MMP), IL-1 $\beta$ , and *vascular endothelial growth factor* (VEGF). The resulting mediator plays a role in increasing vascular permeability.<sup>1</sup>

In this study, there were significantly different levels of MIF between sufferers of DSS and non-DSS. Median levels of MIF in patients with DSS are much higher compared with MIF levels in patients with non-DSS. This result is in accordance with research conducted by Chen LC et al., which found a significant mean difference between the DHF group who died compared with the DHF group that lived.<sup>5</sup> Another study found higher serum MIF levels in severe dengue infection compared with MIF levels in patients with less severe dengue, but not statistically significant.<sup>6</sup> This difference is likely due to sampling problems and different research model used.

MIF levels in the serum of patients with dengue infection were found to increase after day 4 of fever and began to decrease after day 13.<sup>7</sup> Different blood sampling times in the two groups can influence the results of the study. This study used the same method, which is 5-6 days of fever.

The data of this study also showed that mean serum MIF levels increased with increasing DHF grade. A statistically significant was obtained with increasing DHF grade. This data indicates that MIF consistently plays a role in the severity of the degree of DHF. Similar results were also shown by the study of Chen LC et al., and Assunção-Miranda I et al., who found significant differences in the DHF, DF and healthy children groups.<sup>6,16</sup> The weakness of this study is the uneven distribution of samples between each degree of dengue infection, and there has not been analysis of other factors that contribute to the occurrence of severe dengue infection other than the MIF factor only.

## CONCLUSION

Serum MIF levels in patients with non-DSS differ significantly from MIF levels in patients with DSS. *Macrophage Migration Inhibitory Factor* (MIF)

levels serum increase in accordance with the increasing degree of severity of dengue infection. This data shows that MIF has a role in the occurrence of severe dengue infection.

## CONFLICT OF INTEREST

The authors declare that there is no competing interest regarding the manuscript.

## ETHICAL CONSIDERATION

This research was conducted based on the ethical conduct of research from the Ethics Committee of the Medical Faculty, Udayana University/Sanglah Hospital Denpasar No. 1716/UN.14.2/Litbang/2016 and have received permission from the Research and Development Unit (R & D) of Udayana Medical Faculty/Sanglah Hospital with No. LB.02.01/II.C5.D11/14467/2016.

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## AUTHOR CONTRIBUTION

All of the authors are equally contributed to the study from the conceptual framework, data gathering, data analysis, until interpreting the results of the study on publication.

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