

Association of Endothelial Glycocalyx and Tight and Adherens Junctions With Severity of Plasma Leakage in Dengue Infection

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Background. The role of vascular endothelial (VE) components in dengue infection with plasma leakage is unknown. Therefore, we conducted a study to determine the adjusted association of the endothelial glycocalyx layer (EGL) and tight and adherens junction markers with plasma leakage.

Methods. A prospective observational study was conducted at Cipto Mangunkusumo Hospital and Persahabatan Hospital, Jakarta, Indonesia. Adult dengue patients admitted to the hospital on the third day of fever from November 2013 through August 2015 were included in the study. Multiple regression analysis was used to determine the adjusted association of the VE biomarkers with the severity of the plasma leakage.

Results. A total of 103 dengue-infected patients participated in the study. In the critical phase, levels of syndecan-1 (odds ratio [OR] = 1.004; 95% confidence interval [CI] = 1.001–1.007) and chondroitin sulfate (OR = 1.157; 95% CI = 1.025–1.307) had an adjusted association with plasma leakage, whereas levels of syndecan-1 (OR = 1.004; 95% CI = 1.000–1.008) and claudin-5 (OR = 1.038; 95% CI = 1.004–1.074) had an adjusted association with severe plasma leakage.

Conclusions. In dengue-infected patients, elevated levels of syndecan-1 and chondroitin sulfate are strongly associated with plasma leakage, and elevated levels of syndecan-1 and claudin-5 are strongly associated with severe plasma leakage.

Keywords. chondroitin sulfate; claudin-5; dengue infection; plasma leakage; syndecan-1.

Plasma leakage is the pathological hallmark of dengue hemorrhagic fever (DHF), which is responsible for the development of severe dengue and dengue shock syndrome (DSS) [1]. This leakage can occur in the febrile phase within 3 days after fever onset but is most commonly detected in the critical phase (ie, 1 day after defervescence) [2, 3]. Plasma leakage is primarily caused by alteration of the permeability of the microvascular endothelium, resulting in movement of plasma albumin and intravascular fluid toward the extravascular space [4, 5]. This movement causes hypoalbuminemia and hypovolemia [4, 6]. The degree of hypoalbuminemia can be used as a marker of vascular endothelial (VE) permeability alteration and the severity of intravascular volume depletion in dengue-infected patients [7, 8]. Previous studies have reported that severe intravascular

volume depletion caused by a severe increase in vascular permeability, which can be associated with a serum albumin level ≤ 3 g/dL, is associated with complications and more severe dengue compared with dengue without plasma leakage or dengue fever (DF) [9, 10].

Plasma protein and solute transport across the endothelium can occur through both transcellular and paracellular pathways [11, 12]. Under normal conditions, plasma proteins such as albumin are transported through the transcellular pathway by the process of transcytosis, whereas small molecules, including urea and glucose, are transported through the paracellular pathway. The transcellular pathway is highly associated with the endothelial glycocalyx layer (EGL), which plays an important role in maintaining vascular integrity. The 4 major components of the EGL are syndecan-1, hyaluronic acid, chondroitin sulfate, and heparan sulfate [13]. By contrast, the paracellular pathway is highly associated with interendothelial junctions. Tight junctions and adherens junctions are the 2 most important components in the control of endothelial permeability [14]. Claudins appear to be a major structural component of tight junctions, and among the various types of claudins, claudin-5 is predominantly expressed in the VE cells [15]. Meanwhile, VE cadherin–catenin complexes are the primary component of adherens junction [16].

In vitro studies using endothelial cell models have reported that dengue virus (DENV) is able to bind to the EGL, decreasing

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the expression of and redistributing VE-cadherin and the tight junction protein ZO-1, which in turn result in increased endothelial permeability [17, 18]. Studies in pediatric patients showed that the serum level of hyaluronan in DSS was significantly higher than that in DF in the acute phase and that urinary heparan sulfate was significantly higher in individuals with DSS compared with healthy subjects [19, 20]. These in vivo studies indicate roles for hyaluronan and heparan sulfate in the pathogenesis of vascular leakage. In addition, a recent study reported that the anti-inflammatory cytokine interleukin 10 (IL-10) and chemokine (C-X-C motif) ligand 10/interferon γ -inducible protein 10 (CXCL10/IP10) play important roles in the induction of vascular leakage [21]. However, existing publications have not assessed the strength of the association of the VE components (ie, EGL and tight and adherens junction markers) with the severity of plasma leakage.

Knowledge of the association of the VE components, cytokines, and chemokines with the severity of plasma leakage is essential to be able to assess the mechanisms underlying microvascular leakage. Therefore, we conducted a study to investigate the adjusted association of the VE components in DF, DHF, and dengue with severe plasma leakage in the febrile and critical phases. We also investigated the association of IL-10 and CXCL10/IP10 with the severity of plasma leakage in the critical phase. These findings provide new insight into the pathophysiology of plasma leakage in dengue-infected patients.

METHODS

Study Design and Population

We performed a prospective observational study at Cipto Mangunkusumo and Persahabatan Hospitals, Jakarta, Indonesia. Patients with dengue infection who were aged >14 years who were admitted to the hospital on the third day of fever from November 2013 through August 2015 were included in the study. Patients with dengue infection were diagnosed based on clinical manifestation of acute fever and a positive result for dengue nonstructural protein 1 (NS1) antigen, as confirmed by both conventional reverse-transcriptase polymerase chain reaction (RT-PCR) and Simplexa Dengue real-time RT-PCR assay [22, 23]. The exclusion criteria included patients with comorbidities and pregnant women.

Clinical and Laboratory Parameters

The clinical characteristics and laboratory parameters of each patient were recorded at the ward before enrollment. The subjects were classified as having plasma leakage based on World Health Organization criteria (ie, an increase in hematocrit >20%, hypoalbuminemia, or the presence of pleural effusions/ascites detected by ultrasonography) [24]. Subjects without plasma leakage were categorized as having DF (group 1). Subjects with plasma leakage and a serum albumin level >3 g/dL were categorized as having DHF (group 2). Subjects with

plasma leakage and a serum albumin level \leq 3 g/dL were categorized as having severe plasma leakage (group 3). Albumin measurement and abdominal ultrasonography were performed 24 hours after defervescence [2, 4].

Detection of the Biomarkers Using Enzyme-Linked Immunosorbent Assay

Biomarkers of the VE components were measured in duplicate using the following commercially available enzyme-linked immunosorbent assays (ELISAs), performed according to the manufacturers' recommendations: human sCD138 (Syndecan-1) ELISA (Diacione), human heparan sulfate ELISA (MyBioSource), human chondroitin sulfate ELISA (MyBioSource), human hyaluronan quantikine ELISA (R&D Systems), human claudin 5 ELISA (MyBioSource), and human VE-Cadherin quantikine ELISA (R&D Systems). Serum samples were diluted according to the manufacturers' recommendation to obtain the best dilution factors to generate an optical density within the range of detection of the ELISA reader. The measurements were performed using a Multiskan microplate reader with a wavelength set to 450 nm. Biomarkers of VE components in all 3 groups were measured twice: on the third day of fever (febrile phase) and 24 hours after defervescence (critical phase). Interleukin 10 and CXCL10/IP10 detection using ELISA kits (both from R&D Systems).

Ethics

The study was approved by the Faculty of Medicine Universitas Indonesia Ethics Committee, and written informed consent was obtained from all patients.

Statistical Analysis

The sample size calculation was performed by estimation of the heparan sulfate population variance as 3.24 [25], which was the highest population variance among all of the VE biomarkers studied. Assuming a difference in the population mean of 1.25, with a confidence level of 95% and a power of 80%, the minimal total sample size required for the 3 groups was 99 patients.

A bivariate analysis with a Mann-Whitney test for nonparametric data regarding the VE biomarkers and the cytokine and chemokine studied was performed between pairs among the 3 groups. The VE biomarker variables that significantly differed based on the severity of plasma leakage in the bivariate analysis were then entered into a multivariate analysis to determine the adjusted association. The statistical analyses were performed using SPSS Version 20.0 and GraphPad Prism version 7.00 for Windows.

RESULTS

Clinical Characteristics

This study enrolled 103 patients with dengue infection. A total of 30 patients (29.1%) were classified as having DF, 50 patients (48.5%) were classified as having DHF, and 23 patients (22.3%)

Table 1. Dengue-Infected Patients: Comparison of Patients With Dengue Fever, Dengue Hemorrhagic Fever, and Severe Plasma Leakage

Variable	DF group (n = 30)	DHF group (n = 50)	Severe plasma leakage group (n = 23)
Sex, no. male/female	14/16	25/25	9/14
Age, y, median (IQR)	21 (19–28)	22 (18–29)	22 (18–30)
Dengue serotype, no. (%)			
DENV-1	6 (20)	13 (26)	5 (21.7)
DENV-2	11 (36.7)	15 (30)	9 (39.1)
DENV-3	8 (26.7)	14 (28)	7 (30.4)
DENV-4	5 (16.7)	8 (16)	2 (8.7)
Degree of hemoconcentration, %, median (IQR)	10.67 (7.26–12.89)	19.20 (13.77–24.39)	20.59 (16.67–31.25)
Albumin concentration, g/dL, median (IQR)	3.69 (3.52–3.75)	3.4 (3.3–3.61)	2.9 (2.8–2.99)
Platelet count × 1,000/μL, median (IQR)	63 (46.5–95.5)	38 (24–60.5)	19 (13–30)
Pleural effusion or ascites, no. (%)	0	34 (68)	20 (87)

Abbreviations: DENV, dengue virus; DF, dengue fever; DHF, dengue hemorrhagic fever; IQR, interquartile range.

were classified as having dengue with severe plasma leakage. **Table 1** presents the clinical characteristics of each study group.

Plasma Concentrations of the Vascular Endothelial Biomarkers

In the bivariate analysis in the febrile phase, there were significantly increased levels of syndecan-1 ($P = .002$), chondroitin sulfate ($P = .007$), and hyaluronan ($P = .03$) in the DHF group compared with the DF group. Levels of syndecan-1 ($P < .001$), chondroitin sulfate ($P = .02$), and hyaluronan ($P = .002$) were significantly higher in the group with severe plasma leakage compared with the DF group as well. We also found a

significantly increased level of claudin-5 ($P = .002$) in the severe plasma leakage group. Between the DHF and severe plasma leakage groups, there were significant differences in levels of syndecan-1 ($P = .04$) and claudin-5 ($P = .04$) (**Table 2**). In the multivariate analysis of the biomarkers, there was an adjusted association for syndecan-1 between the DF and DHF groups as well as between the DF and severe plasma leakage groups. There was also an adjusted association for claudin-5 between the DHF and severe plasma leakage groups (**Table 3**).

In the critical phase, we found significantly different levels of syndecan-1, chondroitin sulfate, and hyaluronan ($P < .001$ for each comparison) between the DF and DHF groups. Between the DF and severe plasma leakage groups, there were significantly different levels of syndecan-1, chondroitin sulfate, hyaluronan, and claudin-5 ($P < .001$ for each comparison). Furthermore, between the DHF and severe plasma leakage groups, we found significantly different levels of syndecan-1 ($P = .03$) and claudin-5 ($P < .001$) (**Table 2**). In the multivariate analysis between the DF and DHF groups, we found an adjusted association for syndecan-1 and chondroitin sulfate. Between the DF and severe plasma leakage groups, we found an adjusted association for syndecan-1 and claudin-5. Finally, between the DHF and severe plasma leakage groups, we found an adjusted association for claudin-5 (**Table 4**).

Cytokine and Chemokine Plasma Concentrations

We found a significantly increased ($P = .03$) IL-10 in the DHF group (median = 9.47 pg/mL; interquartile range [IQR] = 7.65–13.83) compared with the DF group (median = 7.29 pg/mL; IQR = 6.29–10.36) and also in the severe plasma leakage group (median = 11.23 pg/mL; IQR = 8.06–17.06) compared with the

Table 2. Plasma Concentration of the Endothelial Glycocalyx Layer and Tight and Adherens Junction Markers Measured in the Febrile and Critical Phases

Phase of dengue	Biomarker	DF group (n = 30)	DHF group (n = 50)	Severe plasma leakage group (n = 23)
Febrile phase				
	Syndecan-1, ng/mL	30.26 (13.65–159.45)	122.81 (32.46–313.62) ^a	256.42 (100.73–414.09) ^{a, b}
	Heparan sulfate, pg/mL	148.34 (85.14–399.51)	147 (104.53–523.72)	200.93 (112.88–300.71)
	Chondroitin sulfate, ng/mL	24.56 (20.21–34.58)	34.77 (24.93–44.05) ^a	34.62 (24.42–42.62) ^a
	Hyaluronan, ng/mL	207.43 (56.35–553.1)	842.91 (105.48–3025.74) ^a	1216 (320.2–4229) ^a
	Claudin-5, pg/mL	17.42 (11.71–34.56)	28.77 (11.87–52.98)	50.1 (22.7–78.7) ^{a, b}
	VE-cadherin, ng/mL	23.01 (19.29–38.69)	22.81 (19.48–30.01)	22.36 (18.85–29.14)
Critical phase				
	Syndecan-1, ng/mL	35.91 (15.15–160.21)	264.67 (110.58–471.81) ^a	468.87 (278.7–538.06) ^{a, b}
	Heparan sulfate, pg/mL	146.65 (89.15–402.73)	131.42 (86.67–309.58)	148.05 (111.45–217.62)
	Chondroitin sulfate, ng/mL	21.07 (19.07–24.33)	25.91 (22.89–29.04) ^a	28.62 (25.78–31.15) ^a
	Hyaluronan, ng/mL	218.71 (67.07–1792.90)	2720.50 (911.62–4790.03) ^a	3230.31 (2361.29–6617.7) ^a
	Claudin-5, pg/mL	23.77 (11.66–35.31)	20.01 (10.77–48.13)	81.12 (39.12–118.47) ^{a, b}
	VE-cadherin, ng/mL	23.51 (18.09–40.05)	25.06 (20.58–33.42)	31.49 (22.34–41.1)

Data presented as median (interquartile range) for each group. The Mann–Whitney test was used to assess differences between two groups.

Abbreviations: DF, dengue fever; DHF, dengue hemorrhagic fever.

^aSignificant difference from the DF group ($P < .05$).

^bSignificant difference from the DHF group ($P < .05$).

Table 3. Multivariate Analysis of the Plasma Concentrations of the Endothelial Glycocalyx Layer and Tight and Adherens Junction Markers Measured in the Febrile Phase Between Groups

Groups	Biomarker	Odds ratio (95% CI)	P value
DF vs DHF	Syndecan-1	1.005 (1.001–1.009)	.01
DF vs severe plasma leakage	Syndecan-1	1.009 (1.004–1.015)	.001
DHF vs severe plasma leakage	Claudin-5	1.019 (1.001–1.037)	.04

Abbreviations: CI, confidence interval; DF, dengue fever; DHF, dengue hemorrhagic fever.

DF group ($P = .02$) (Figure 1). Moreover, there was a significantly increased ($P = .01$) level of CXCL10/IP10 in the DHF group (median = 46.07 pg/mL; IQR = 26.02–56.84) compared with the DF group (median = 23.69 pg/mL; IQR = 17.78–41.51). Finally, there was a significantly increased ($P = .006$) level of CXCL10/IP10 in the severe plasma leakage group (median = 61.39 pg/mL; IQR = 22.24–108.13) compared with the DF group (Figure 2).

DISCUSSION

This is the first Indonesian study to assess the role of EGL and endothelial cell junction components in determining the severity of plasma leakage, which is responsible for the life-threatening nature of DHF. Hypoalbuminemia is objective evidence of plasma leakage due to vascular permeability alteration [24]. Previous studies reported that the level of serum albumin was associated with the severity of dengue infection and could be used as a surrogate indicator of severe plasma leakage [8, 9].

Bivariate analysis of EGL components in the febrile phase showed significantly increased levels of syndecan-1, chondroitin sulfate, and hyaluronan in the DHF and severe plasma leakage groups compared with the DF group. An explanation for these findings is that there are several possible mechanisms of vascular permeability alteration due to EGL degradation in dengue infection based on previous *in vitro* studies. The first mechanism is release of cytokines and chemokines by peripheral blood mononuclear cells through direct activation of Toll-like receptor 4 by NS1 [26]. The second mechanism is complement activation induced by either attachment of NS1 to the glycosaminoglycans heparan sulfate and chondroitin sulfate E or binding of DENV to a specific syndecan-2 proteoglycan receptor [27–29]. The third mechanism is activation of endothelial cell-intrinsic pathways caused by binding of DENV NS1 to the glycocalyx [26]. These 3 possible mechanisms may explain the degradation and shedding of the EGL components in the circulation [26, 29]. However, we found no significant increase in the level of heparan sulfate within the 3 groups. Furthermore, in the multivariate analysis, the only EGL component that had a strong association with the severity of plasma leakage was syndecan-1. Our finding may be explained based on the mechanism that regulates synthesis of the syndecan-1

Table 4. Multivariate Analysis of the Plasma Concentrations of the Endothelial Glycocalyx Layer and Tight and Adherens Junction Markers Measured in the Critical Phase Between Groups

Groups	Biomarker	Odds ratio (95% CI)	P value
DF vs DHF	Syndecan-1	1.004 (1.001–1.007)	.02
	Chondroitin sulfate	1.157 (1.025–1.307)	.02
DF vs severe plasma leakage	Syndecan-1	1.004 (1.000–1.008)	.047
	Claudin-5	1.038 (1.004–1.074)	.03
DHF vs severe plasma leakage	Claudin-5	1.022 (1.009–1.036)	.001

Abbreviations: CI, confidence interval; DF, dengue fever; DHF, dengue hemorrhagic fever.

core protein by heparan sulfate. In endothelial cells, DENV NS1 induces lysosomal cysteine proteinase and activates heparanase through enzymatic cleavage [26]. Heparanase then cleaves heparan sulfate chains, resulting in degradation of the heparan sulfate attached to syndecan-1. Diminished levels of heparan sulfate chains on the syndecan-1 core protein cause a significant increase in shedding of syndecan-1 [30]. This effect is specifically induced by NS1 from DENV-1–4, and not by proteins from other flaviviruses [26].

In this study, both bivariate and multivariate analyses of tight junction markers showed an adjusted association for claudin-5 between the DHF and severe plasma leakage groups. This finding is consistent with an *in vitro* study that showed disrupted tight junctions among human VE cells after exposure to monocyte chemoattractant protein-1 (MCP-1) from the sera of DSS patients [31]. Bivariate analysis of adherens junction

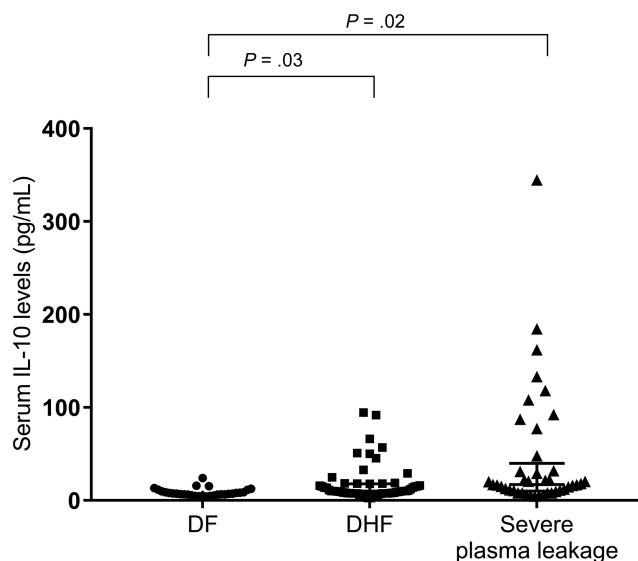


Figure 1. Scatter plot of the serum interleukin 10 (IL-10) levels in the dengue fever (DF), dengue hemorrhagic fever (DHF), and severe plasma leakage groups. The horizontal bars represent the median serum IL-10 levels. Differences in the median between pairs of groups were tested using the Mann–Whitney test ($P = .03$ for the difference between the DF and the DHF groups; $P = .02$ for the difference between the DF and the severe plasma leakage groups).

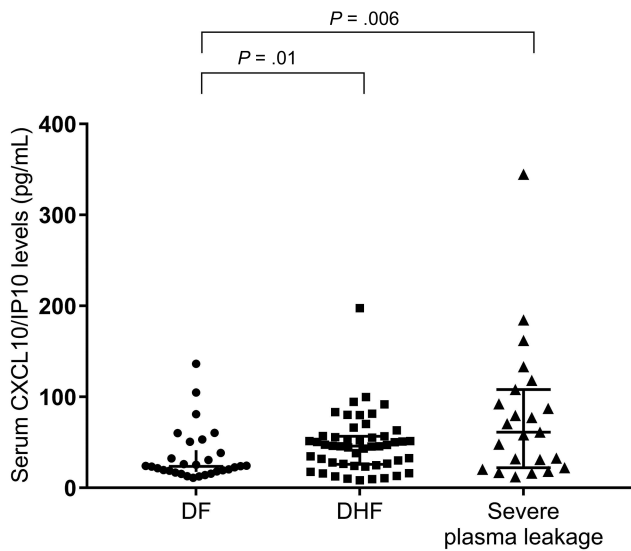


Figure 2. Scatter plot of the serum chemokine (C-X-C motif) ligand 10/interferon γ -inducible protein 10 (CXCL10/IP10) levels in the dengue fever (DF), dengue hemorrhagic fever (DHF), and severe plasma leakage groups. The horizontal bars represent the median serum CXCL10/IP10 levels. Differences in the median between pairs of groups were tested using the Mann–Whitney test ($P = .01$ for the difference between the DF and the DHF groups; $P = .006$ for the difference between the DF and the severe plasma leakage groups).

markers in the febrile phase showed there was no difference in VE-cadherin levels within the subject groups. VE-cadherin has been proposed as a biomarker reflecting endothelial damage. In fact, in patients with hemolytic uremic syndrome (HUS) caused by Shiga toxin–producing *Escherichia coli* (STEC) infection, soluble VE-cadherin levels are significantly higher than in non-HUS patients; this finding indicates that VE-cadherin is released when membrane damage occurs [16]. In contrast with HUS, plasma leakage in dengue infection occurs without morphological damage to the vascular endothelium [29]. This feature may explain the lack of a difference in VE-cadherin levels within the groups of dengue patients with different severities of plasma leakage in this study. However, further studies are needed to determine the role of VE-cadherin in the most severe dengue infections, such as in DSS. Our findings regarding biomarker levels in the febrile phase suggest that syndecan-1 plays a dual role as a risk factor for plasma leakage occurring at 24 days after defervescence: first, as a risk factor for plasma leakage in general, and second, as a risk factor for severe plasma leakage together with claudin-5.

As has been discussed previously, syndecan-1 plays a vital role in the early phase, serving as a risk factor for plasma leakage, and this role has also been confirmed in the critical phase. We specifically found an adjusted association for syndecan-1 between the DF and DHF groups. We also found significantly elevated chondroitin sulfate in the DHF group compared with the DF group. However, based on the multivariate analysis, the adjusted association of elevated chondroitin sulfate only

persisted in the critical phase. This finding can be explained by the fact that plasma leakage is most commonly detected in the critical phase [2, 3]. In addition, the median level of chondroitin sulfate was lower in the critical phase compared with the febrile phase. A previous study characterizing circulating glycosaminoglycans during critical illness reported a decline in circulating chondroitin sulfate 72 hours after study enrollment; this finding may reflect glycosaminoglycan clearance by the liver or kidney [32].

Physiologically, syndecan-1 contains 3 attachment sites for heparan sulfate and 2 additional attachment sites for chondroitin sulfate [33]. We postulate that the peak shedding of syndecan-1 is followed by a significant increase in the level of chondroitin sulfate. In contrast, due to heparanase activity, there is no increase in the level of heparan sulfate [30]. Although hyaluronan showed a significant association in the bivariate analysis in both the febrile and the critical phases, the association did not persist in the multivariate analysis. This finding may indicate a less significant role for hyaluronan, which is located on the apical surface of endothelial cells [33], compared with syndecan-1 and chondroitin sulfate in terms of the process of plasma leakage in dengue infection. In vitro studies have reported that hyaluronidase treatment to remove hyaluronan results only in minimal changes in permeability. It appears that the components of the EGL other than hyaluronan can stabilize the properties of the glycocalyx [34].

We found significantly increased levels of the cytokine IL-10 and the chemokine CXCL10/IP10 in the DHF group compared with the DF group in the critical phase. A previous study in pediatric patients reported that circulating levels of IL-10 and CXCL10/IP10 significantly increased in dengue patients with vascular leakage [21]. A study in hemorrhagic shock patients reported a significantly increased level of syndecan-1 after injury and a positive correlation between the IL-10 level and syndecan-1 shedding. Furthermore, an in vitro model of endothelial injury showed that syndecan-1 shedding correlated with changes in endothelial permeability [35], and another in vitro study revealed that chemokines stimulate cell metalloproteinases, which are involved in syndecan shedding [36].

We found no significant association between DF and DHF and either tight or adherens junction components in the critical phase. The glycocalyx plays an important role as an endothelial barrier because of its net negative charge. This role is supported by experiments that characterized the effect of disrupting the glycocalyx or neutralizing its negative charge [11]. In particular, treatment with neuraminidase, causing removal of the majority of the glycocalyx, increased albumin flux across the endothelium [37]. Moreover, neutralization of the apical endothelial negative charge with cationic agents (eg, ferritin or protamine) increased the transendothelial permeability of albumin [11]. The transcellular pathway is initiated by binding of albumin to the protein gp60, which induces the phosphorylation of the Src

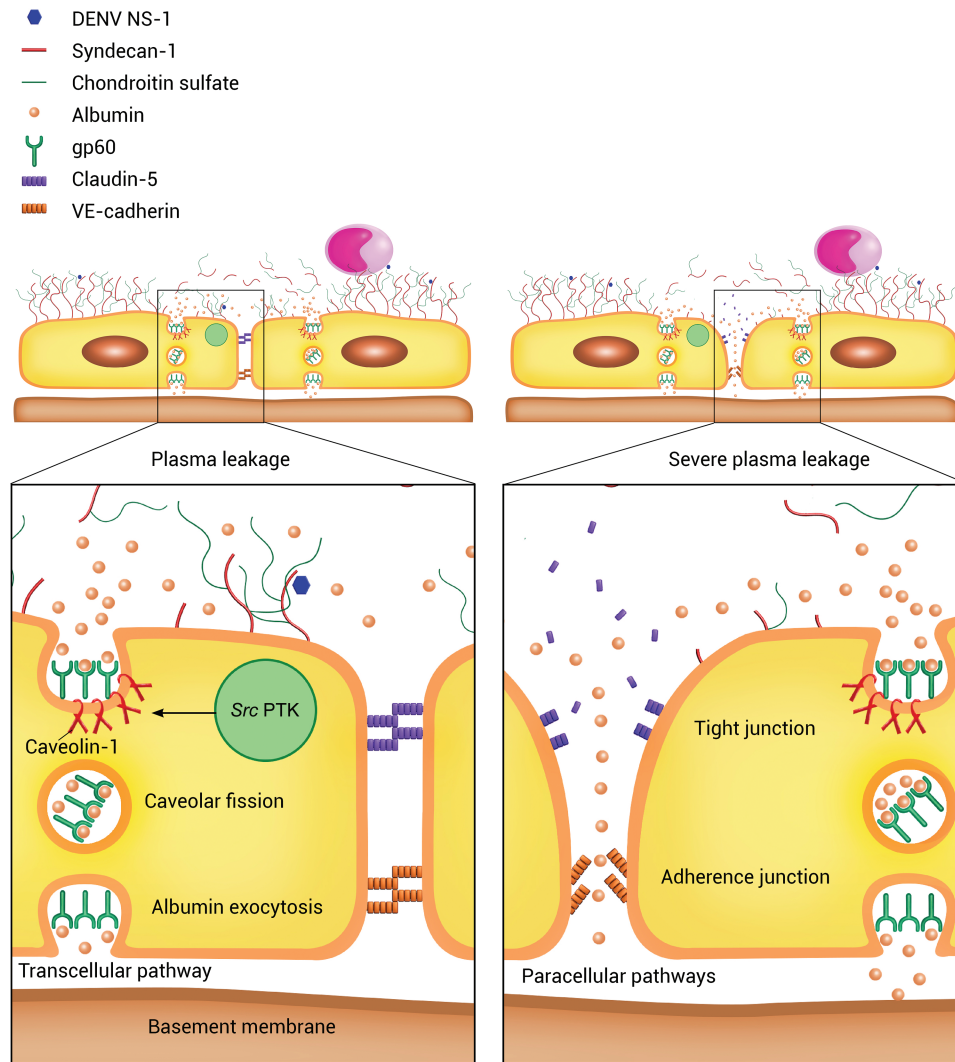


Figure 3. Proposed mechanism of microvascular leakage in dengue infection. Plasma leakage: dengue virus (DENV) nonstructural protein 1 (NS1)-induced degradation of syndecan-1 and chondroitin sulfate results in gp60 activation by albumin. Gp60 activation leads to phosphorylation of the *Src* protein tyrosine kinases (PTKs) and caveolin-1, which is responsible for regulation of caveolar fission. Cytokines and chemokines can also stimulate activation of *Src* PTKs. This process increases albumin exocytosis through the transcellular pathway. Severe plasma leakage: Claudin-5 degradation causes retraction of endothelial cells and opening of intercellular gaps, allowing albumin movement into the extravascular space through paracellular pathways. Albumin leakage that occurs through both paracellular and transcellular pathways results in severe plasma leakage.

protein tyrosine kinases (PTKs), leading to phosphorylation of caveolin-1 and dynamin-2. The activation of these proteins is required for caveolar fission and the internalization of albumin within caveolae [38], and inflammatory cytokines such as TNF- α may directly increase the phosphorylation of the *Src* PTKs [39]. Moreover, an in vitro study using human dendritic cells showed that chemokines could stimulate the activation of *Src* PTKs in both immature and mature dendritic cells [40]. Another in vitro study found that DENV infection enhances caveolae-mediated albumin transcytosis, which consequently induces vascular leakage [41]. These mechanisms may explain the increase in albumin leakage through transcytosis in DHF, independent of the paracellular pathway.

Consistent with our findings in the febrile phase, in the critical phase, there was an adjusted association for syndecan-1 and claudin-5 between the DF and severe plasma leakage groups. We also found that claudin-5 had an adjusted association between the DHF and the severe plasma leakage groups. In addition, there were increased levels of IL-10 and CXCL10/IP10 in the severe plasma leakage group compared with the DF group. The disruption of tight junctions has been elucidated by in vitro studies that have determined that the mechanism of severe dengue is based on excessive release of various cytokines and chemokines, known as cytokine storm, as observed in U937-derived macrophages infected with a humanized monoclonal antibody recognizing protein E [42].

This degradation of tight junctions allows the passage of albumin across the endothelial barrier, causing hypoalbuminemia, even without degradation of adherens junctions [11]. The potential explanation for this paracellular pathway is that endothelial-cell retraction may lead to opening of intercellular gaps. This reversible effect may be caused by histamine, thrombin, and VE growth factors, without affecting vascular damage [11, 43]. In sum, the results reflect major roles for syndecan-1 and claudin-5 in the mechanism of albumin leakage through transcellular and paracellular pathways during severe plasma leakage. A proposed mechanism of microvascular leakage in dengue-infected patients based on the overall results of our study is shown in Figure 3.

A limitation of our study is the measurement of EGL components and tight and adherens junction markers in the plasma. In particular, the predominant source of these components is endothelial cells, but these components are also present on epithelial tissues and macrophages, so the levels of these components in the circulation might not be solely influenced by endothelial cells.

In conclusion, in patients with dengue infection, elevated levels of syndecan-1 and chondroitin sulfate have a strong association with plasma leakage, and elevated levels of syndecan-1 and claudin-5 have a strong association with severe plasma leakage.

Notes

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