

Major Article

Upregulation of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in renal tissue in severe dengue in humans: Effects on endothelial activation/dysfunction

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Abstract

Introduction: Dengue is an important mosquito-borne disease in tropical and subtropical regions. Adhesion molecules have not been systematically characterized in the renal tissue of patients with severe dengue (SD). The objective of this study was to detect viral antigens in samples from patients that evolved with SD, correlating with the expression of ICAM-1, VCAM-1, VE-cadherin, and E-selectin to contribute to a better understanding of the pathophysiology of SD. **Methods:** Kidney specimens from patients with SD were selected according to clinical and laboratorial data and submitted to histological and immunohistochemistry analysis. A semiquantitative evaluation was performed considering positive immunostaining in 20 glomeruli. **Results:** Viral antigens were mainly detected in distal tubules. The intense immunostaining of VCAM-1 and ICAM-1 was observed. The expression of E-selectin was discrete, and VE-cadherin expression varied from mild to moderate. VCAM-1 was slightly intense in the glomerular capsule; the expression of ICAM-1 was diffuse. E-selectin was diffuse, and VE-cadherin varied from mild to moderate. The most frequent histological findings were glomerular congestion, mild glomerulitis, acute renal injury, and glomerular atrophy. **Conclusions:** The results appear to demonstrate an imbalance between vascular endothelial permeability regulating events in renal lesions in SD. The increase in the expression of ICAM-1 and VCAM-1 is an *in-situ* indicator of higher permeability with a consequent influx of cells favoring the inflammation of the endothelium. These molecules are important in the pathophysiology of the disease and provide the possibility of developing new markers for the evaluation, clinical follow-up, and therapeutic response of patients with SD.

Keywords: Adhesion molecules. Kidney. Dengue. Immunopathogenesis.

INTRODUCTION

Dengue is one of the most prominent mosquito-borne viral diseases in tropical and subtropical regions. This disease presents various clinical manifestations, classified as dengue without warning signs, dengue with warning signs, and severe

dengue (SD)¹⁻⁵. For SD, endothelia potentially undergo intense changes contributing to the pathogenesis of the hemorrhagic phenomena observed in the clinical course. In this context, endothelial cells can be altered via two distinct mechanisms: (1) by the action of soluble factors released primarily by host cells causing activation and endothelial dysfunction and (2) via direct cytopathic viral effects⁶⁻⁸.

During disease progression, mononuclear phagocytes interact with the primary target of infection via these viruses and are potential producers of proinflammatory cytokines (IL1 β ,

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TNF- α , and IL6), which appear crucial for disease pathogenesis. Such cytokines act on the vascular endothelium by activating intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and vascular endothelial cadherin (VE-cadherin) molecules by altering the endothelial permeability and function and attracting monocytes to their surface for transendothelial migration⁹⁻¹³. VCAM-1 activation increases endothelial permeability and releases intracellular signals, leading to the loss of VE-cadherin adhesion and stress in actin fibers¹⁴.

E-selectin is an endothelial/circulating cell adhesion molecule that mediates signaling and the recruitment of circulating cells. E-selectin participates in transmembrane motion, and its downregulation or absence in dengue may be one of the factors for the discrete formation of inflammatory infiltrate in the liver. When assessing patients with dengue, high serum VCAM-1, ICAM-1, and E-selectin levels suggest activation and damage to endothelial cells¹⁵⁻¹⁷.

A previous study has reported the expression of different markers of cellular phenotype, cytokines, and endothelial activation during liver injury in cases of SD¹⁷. Upon the analysis of renal tissue, another important organ in the study of SD, an *in situ* immune response was detected, comprising lymphomononuclear infiltrate with a predominance of CD4⁺ T cells and an excessive number of macrophages and cells expressing IL-17 and IL-18, thereby potentially increasing vascular permeability¹⁸. IL-17, primarily produced by CD4⁺ T lymphocytes, in addition to fibroblasts and endothelial cells, is an important factor of the adaptive immune response to several infectious agents, including the dengue virus (DENV). In addition to upregulating ICAM-1 in the endothelium, fibroblasts, and epithelial cells, these cells stimulate the secretion of IL-6, IL-8, GM-CSF, and PGE2^{19,20}.

Analysis of the immunopathogenesis of viral hemorrhagic fever, particularly SD, showed that the liver and kidneys are the preferred target organs of the virus, and lesions were observed with an increase in disease severity. In addition, hemorrhagic

phenomena observed in these cases was partly due to endothelial involvement and alteration, which may be induced directly by viruses or soluble factors released during infection. Some studies have already characterized the cellular and cytokine immune response profile in cases of SD. However, additional data, including the expression of adhesion molecules, have not yet been systematically characterized in the renal tissues of patients with SD. This study aimed to detect the viral antigens of the DENV in renal samples from patients experiencing SD progression, and correlate the data with ICAM-1, VCAM-1, VE-cadherin, and E-selectin expression to further the current understanding of disease pathophysiology.

METHODS

Samples

Ten formalin-fixed paraffin-embedded kidney specimens from individuals who died due to dengue were selected from the archives of the Department of Pathology at the Hospital Guilherme Álvaro, municipality of Santos, São Paulo State, Brazil. The patients' ages ranged from 4-62 years. Cases were selected in accordance with clinical and laboratory data and serological diagnosis.

Histological and immunohistochemical analysis

The specimens were formalin-fixed, paraffin-embedded, and stained in hematoxylin-eosin (HE) for histological evaluation. Samples were hydrated in a series of ethanol grades. Endogenous peroxidase activity was blocked by incubating sections in 3% H₂O₂ solution for three incubations of 10 min each. Sections were incubated in an antigen retrieval solution (Dako, S2367) at pH 9.0 for 20 min at 95°C. Immunohistochemical staining was performed via incubation with specific primary antibodies (**Table 1**). Tissue samples were incubated with a secondary antibody and a streptavidin-biotin peroxidase system, in accordance with the manufacturer's instructions (LSAB system, code K0690, DakoCytomation, Carpinteria, CA, USA) or a protocol based on polymers. Each reaction was developed with 3,3'-diaminobenzidine-tetrahydrochloride

TABLE 1: List of antibodies applied in the immunohistochemistry protocol, with mark, code, and work dilution.

Antibody	Mark/Code	Work dilution
Polyclonal anti-dengue virus type II	Courtesy of Instituto Evandro Chagas – Para – Brazil	1:100
Monoclonal mouse anti-ICAM-1	Novocastra/NCL-CD54-307	1:50
Polyclonal goat anti-VCAM-1	RD Systems/BBA19	1:200
Monoclonal mouse anti-E-selectin	Novocastra/NCL-CD62E-382	1:50
Monoclonal mouse anti-VE-cadherin	Novocastra/NCL-VE-cad	1:50

(DAB) (Sigma-Aldrich Chemical Co., St. Louis, MO, USA D5637). All specimens were counterstained with Mayer's hematoxylin. Positive and negative controls were subjected to each immunohistochemical reaction.

Quantitative analysis of the specimens was performed considering the presence of a positive reaction in 20 randomized glomeruli. The results are expressed as percentages and classified based on scores (0, absent; up to 5 glomeruli with a positive reaction, discrete; 6-10 glomeruli with a positive reaction, moderate; 11-20 glomeruli with a positive reaction, intense).

Ethical aspects

This study was approved by the Ethics Committee of the Medical School, University of São Paulo, and process 253/12.

RESULTS

The most frequent renal histological findings were mild-to-severe glomerular congestion, mild-to-severe glomerulitis acute renal injury, increased capsule-glomerular space due to glomerular atrophy, and hyalinization of glomeruli in some specimens.

Regarding the distribution of viral antigens, the region with the highest predominance of immunostaining was the distal tubules.

For this study, a positive reaction in the glomeruli was given preference because previous data indicate that the primary renal alteration in hemorrhagic dengue is characterized by glomerular involvement.

Quantitative analysis of glomerular expression of the adhesion molecules revealed intense immunostaining of VCAM-1 and ICAM-1 (except in one negative specimen). However, E-selectin expression was discrete in four cases, intense in three, and negative in three, whereas that of VE-cadherin varied from intense to moderate. Furthermore, VCAM-1 expression was slightly higher in the glomerular capsule than that in the glomerular capillary tufts, whereas ICAM-1 expression was diffuse and of similar intensity throughout the glomerular structure (**Figure 1**). **Figure 2** shows the percentage of glomeruli with a positive immunoreaction.

DISCUSSION

VHFs constitute a group of diseases caused by viruses of different families and are clinically characterized by fever and hemorrhagic phenomena, resulting from a severe systemic disorder that progresses in compromised organs. Although caused by different viruses, the pathogenesis of these infections shares a common vascular substrate and preferential lesions in some organs, such as the liver and kidneys²¹. Therefore, during the disease course, the expression of endothelial ligands is altered, which contributes significantly to the systemic progression of this infection and the induction of cellular lesions, especially in the aforementioned organs. ICAMs are expressed in different cell types with different regulatory patterns and effector functions. ICAM-1 is constitutively expressed in small numbers in the vascular endothelium, some lymphocytes, and

monocytes. Some inflammatory cytokines, such as TNF- α , IL-1, and IFN- γ , upregulate these molecules in endothelial cells, accompanied by the subsequent upregulation of other cytokines and adhesion molecules, such as VCAM-1²².

The release of cytokines, such as TNF- α , IL-1, and IFN- γ , during SD, may alter the expression of adhesion molecules, thereby ultimately causing changes in vessels during the infection. Alterations in VE-cadherin expression reflect changes in vascular permeability resulting from pro-inflammatory cytokines which, together with the discrete or moderate increase in E-selectin and VCAM-1, allows for the migration of leukocytes to the glomeruli during the course of glomerulonephritis in these patients and may also induce glomerular hemodynamic changes underlying acute renal failure in patients with SD²³⁻²⁵.

Serum levels of IL-17 and ICAM-1 are higher in dengue with severe clinical manifestations, compared to those presenting a moderate but non-prognostic disease course. It was considered feasible to correlate the increase in serum levels of these markers with the observations obtained from the specimens, due to their positive immunostaining in the renal parenchyma.

ICAM-1 is an endothelial activation marker that binds to T lymphocytes via LFA-1 and Mac-1 ligands. IL-17 is an activator of ICAM-1 and participates in local immune reactions. IL-17 is produced primarily by CD4⁺ T cells; however, epithelial cells, fibroblasts, and endothelial cells also produce IL-17. IL-17 upregulates ICAM-1 in fibroblasts, epithelia, and endothelia, and stimulates the secretion of IL-6, IL-8, GM-CSF, and PGE2 by these cells.

VCAM-1 is also a marker of endothelial activation; it binds to T lymphocytes via VLA-1²⁶. The upregulation of these markers is an *in-situ* indicator of increased permeability with the consequent influx of cells favoring endothelial inflammation. In patients with SD, VCAM-1 is upregulated in the liver, whereas VE-cadherin expression remains unchanged, being similar to healthy organs. Both molecules are expressed in portal spaces and hepatic arteries (unpublished data).

E-selectin, expressed in the vascular endothelium, recruits monocytes and polymorphonuclear cells, and VE-cadherin expression is reportedly altered in dengue, due to functional alterations in the endothelium. Several studies have reported changes in the levels of endothelial activation markers, including VCAMs and ICAMs. An increase in permeability occurs in conjunction with VE-cadherin downregulation, thereby regulating the barrier function of adherens junctions²⁷.

Endothelial cells express IL-17 receptors along with adhesion molecules responsible for leukocyte transmigration. However, IL-17 activates E-selectin, VCAM-1, and ICAM-1 expression²⁸. Acute infection by DENV-2 increases IL-17 blood levels during SD-related mortality¹⁶. A recent study reported a large number of cells expressing IL-17 and IL-18 via immunohistochemical analysis, thereby reflecting the acute inflammatory response and contributing to local lesions, besides indicating that such cytokines favor an increase in vascular permeability¹⁸.

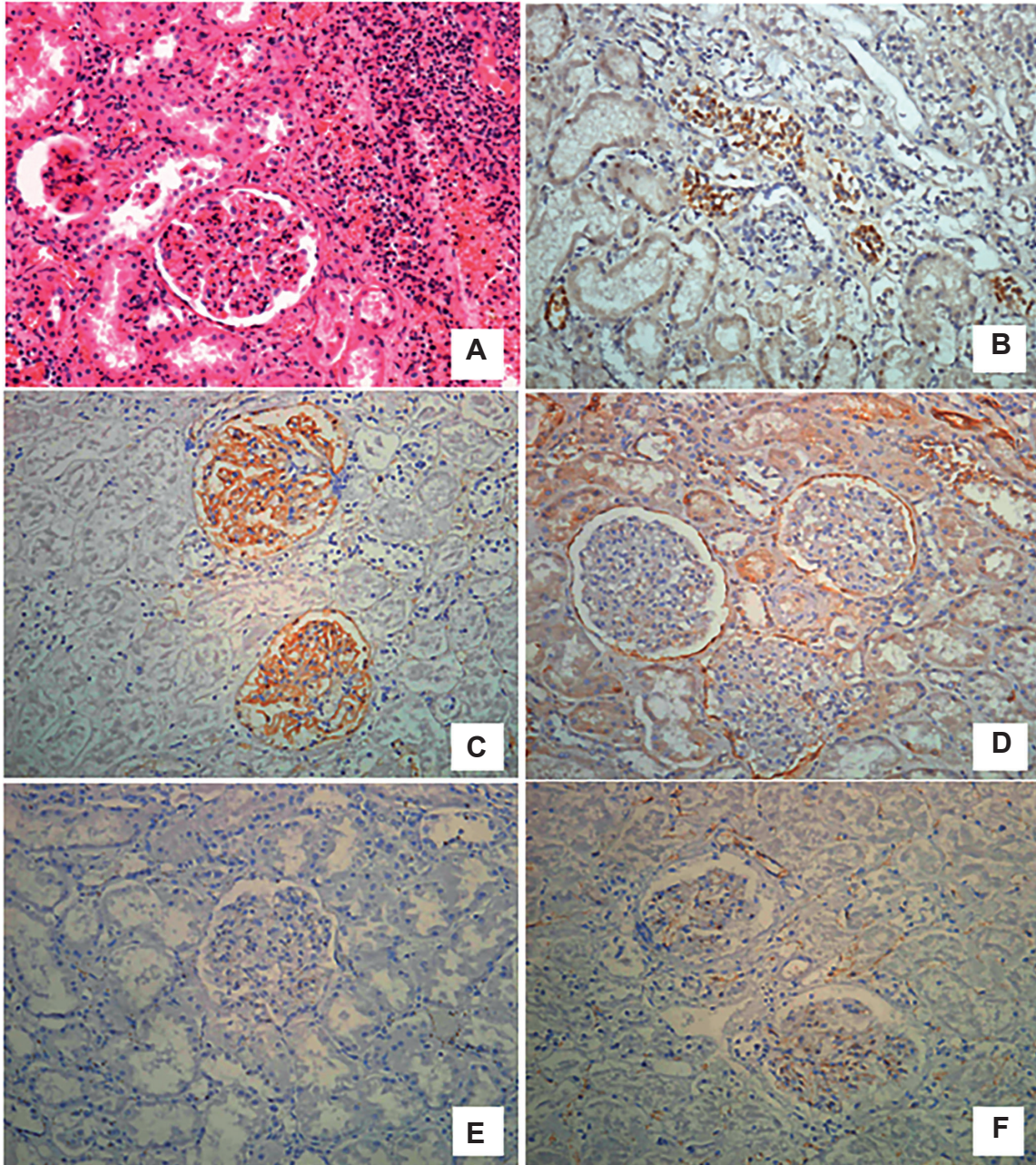


FIGURE 1: (A) Histological analysis of kidney injury in SD presenting glomerular congestion, mononuclear cell infiltration, glomerulitis, and discrete glomerular atrophy (hematoxylin-eosin; 200x). (B) Viral antigens detected by immunohistochemistry in distal tubules. Intense glomerular expression of VCAM-1 (C) and (D) ICAM-1. (E) and (F): Discrete expression of E-selectin and VE-cadherin, respectively (Immunohistochemistry, x200).

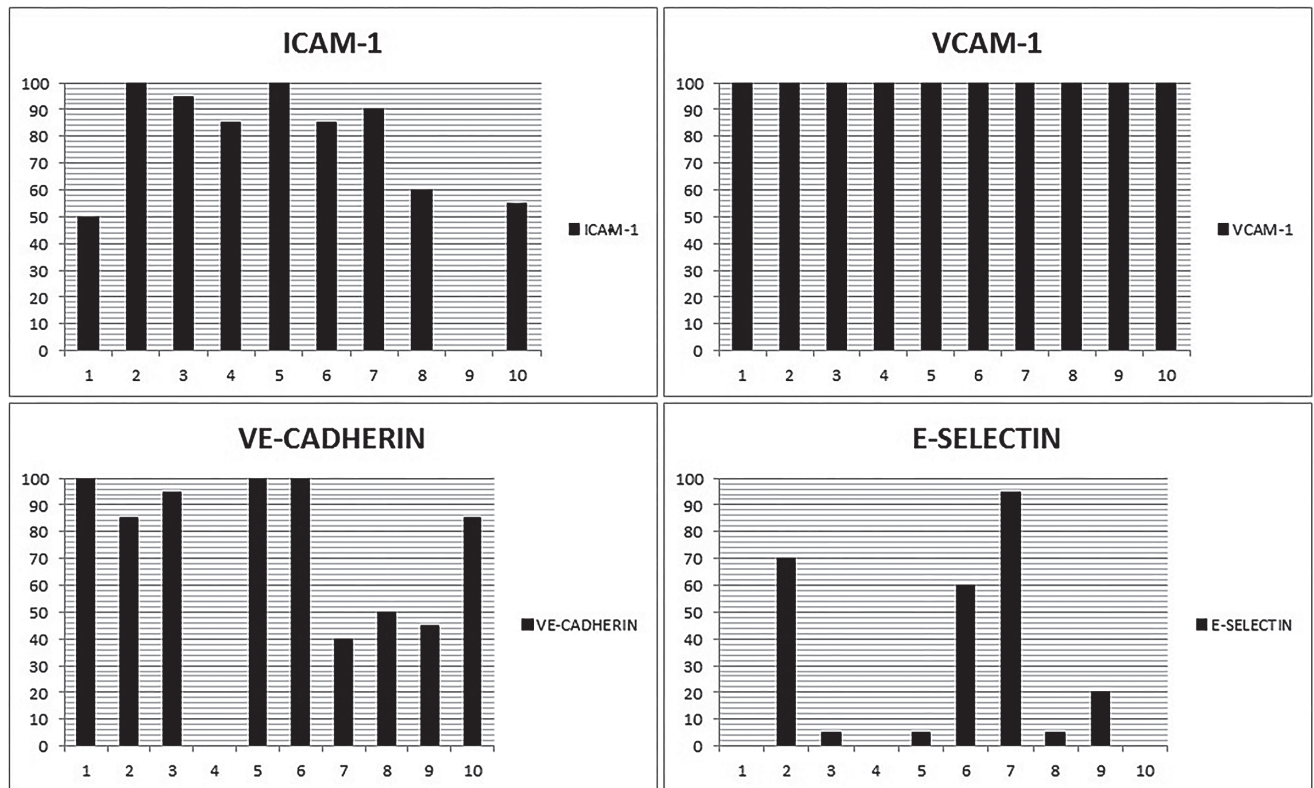


FIGURE 2: Analysis of the renal specimens considering the percentage presence of positive immunostaining in 20 glomeruli, evaluated according to the tissue expression of the components (0 – absent, up to 5 glomeruli (25%) – discrete, 6-10 glomeruli (50%) – moderate, 11-20 glomeruli (100%) – intense).

The association between IL-17 levels and the expression of ligands and endothelial adhesion molecules in SD provides novel avenues regarding the application of cytokines and markers of activation and endothelial alteration as prognostic markers in cases of DENV and other infections resulting in hemorrhagic fevers. During subclinical endothelial activation and the subsequent progression to severe disease, peripheral blood biomarkers of endothelial activation/dysfunction may be useful in identifying patients with the potential for disease progression to the severe form²⁹.

According to the current classification, the previously named dengue hemorrhagic fever is characterized by a transient increase in vascular permeability resulting in plasma leakage, high fever, bleeding, thrombocytopenia, and hemoconcentration, which can lead to shock (termed dengue shock syndrome (DSS)). In 1974, the WHO committee developed classification guidelines based on studies of disease patterns in children in Thailand in the 1960s. The 1997 guidelines classified dengue into DF, DHF (Grades 1 and 2), and DSS (DHF Grades 3 and 4). However, in 2006 the WHO Dengue Scientific Working Group recommended additional research into dengue diagnostics and the triaging of patients for optimized clinical management. The 2009 WHO criteria classified dengue according to levels of severity and SD includes cases involving severe plasma leakage, severe bleeding, or organ failure. The specimens from the present casuistic came from patients who died from the most severe forms of the disease (DHF grades 3 or 4), according to the classifications of 1997^{30,31}.

Finally, the tubular localization of viral antigens is concurrent with previous reports describing antigenic distribution in different human tissues via immunohistochemical analysis and *in situ* hybridization. In the kidney, viral antigens were reported as discrete granular deposits within tubular lining cells, thereby suggesting that they potentially represent reabsorbed immune complexes following renal elimination, as no viral RNA was detected at those sites³².

Therefore, studies similar to the present study reinforce the importance of adhesion molecules in dengue pathophysiology and provide novel avenues regarding the development of new markers for the evaluation, clinical follow-up, and therapeutic response of patients with SD.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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