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Human kidney damage in fatal dengue hemorrhagic fever results of glomeruli injury mainly induced by IL17

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ABSTRACT

Acute kidney injury is an unusual complication during dengue infection. The objective of this study was to better identify the characteristics of glomerular changes focusing on in situ immune cells and cytokines. An immunohistochemical assay was performed on 20 kidney specimens from fatal human cases of dengue hemorrhagic fever (DHF). It was observed a lymphomononuclear infiltrate, neutrophils and nuclear fragmentation in the glomeruli, hydropic degeneration, nuclear retraction, eosinophilic tubules and intense acute congestion. Sickle erythrocytes were frequent in glomeruli and inflammatory infiltrate. The glomeruli presented endothelial swelling and mesangial proliferation. Lymphocytes CD4+ predominated over CD8+ T cells, B cells and natural killer cells. There were also an expressive number of macrophagic CD68+ cells. S100, Foxp3 and CD123 cells were not identified. Cells expressing IL17 and IL18+ cytokines predominated in the renal tissues, while IL4, IL6, IL10, IL13, TNF-alpha and IFN-gamma were rarely visualized. The high number of cells expressing IL17 and IL18+ could reflect the acute inflammatory response and possibly contribute to the local lesion. CD8+ T cells could play a role in the cytotoxic response. DHF is a multifactorial disease of capillary leakage associated with a "Tsunami of cytokines expression". The large numbers of cells expressing IL17 seems to play a role favoring the increased permeability.

1. Introduction

Dengue is the most important viral mosquito-borne diseases affecting humans in tropical and subtropical regions worldwide. The presentation of the infection ranges from asymptomatic infection to severe fatal Dengue Hemorrhagic Fever (DHF), which is characterized by hemorrhagic manifestations, thrombocytopenia, plasma leakage and or shock [18,11,9,24,7].

During fatal DHF, viral antigens have been detected in many tissues and organs including liver, lung, lymph nodes, skin and kidney. An immunohistochemistry Immunohistochemical studies revealed that viral antigens in the kidney are present as granular deposits in the lining cells of the tubules [13,17].

Acute kidney injury is an unusual complication during severe dengue and is associated to hypotension, rhabdomyolysis or hemolysis. However, cases of dengue with acute kidney injury without such characteristics have also been reported. In those cases, the injury is explained as been probably mediated by a direct effect of dengue virus-DENV [21,2].

Most studies concerning renal involvement during the course of dengue have reported considerable rates of mortality [20]. The analysis of renal biopsies demonstrate glomerular changes such as

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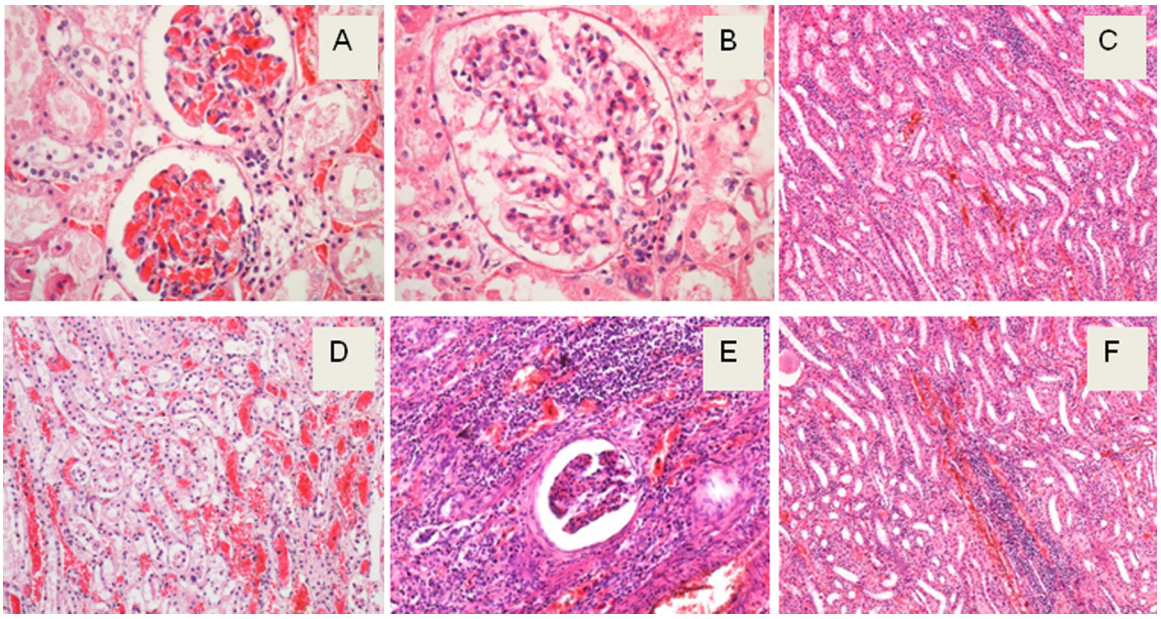

Fig. 1. Histological aspects of kidney injury in cases of dengue hemorrhagic fever. (A) Glomerular congestion with some elongated and angled red blood cells. Some tubule cells have pyknotic nuclei (×400); (B) discrete endothelial proliferation. Acute tubular necrosis (nuclear pyknosis) (×400); (C) chronic tubulointerstitial nephritis. Focal acute tubular necrosis (×200); (D) intense congestion, some tubule cells with pyknotic nuclei, possible tubular necrosis (×200). (E) Glomerular congestion. Some white blood cells in capillary loops; (F) infiltrated tubulointerstitial nephritis, possible chronic pyelonephritis, mononuclear cell infiltration, mild tubular dilation, hyaline casts, congestion of peritubular capillaries (×200).

hypertrophy and hyperplasia of mesangial and endothelial cells, monocyte-like cells in some glomerular capillary lumens and focal thickening of the glomerular basement membrane. In other cases, immune complex deposition is a common histologic feature [8].

Vascular alterations have been also observed and seem to be related to the imbalance of the host immune response, as a consequence of the pattern of cytokines and cells present at the sites of lesions [5].

Although the liver and lungs are considered important target organs of DENV in severe disease, other organs are also injured and matter of studies, such as spleen, heart and kidneys [31,30,3].

Considering the importance of severe dengue (DHF) in the context of public health worldwide, the objective of this study was to better identify the immune and histopathology characteristics of glomerular, tubular and interstitial renal changes focusing on the phenotype of immune cells at that site and their implications to the immunopathogenesis of dengue. The results of our study suggest that glomeruli injury is induced by interleukin 17 (IL17).

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

Antibody	Mark/code	Work dilution
Monoclonal anti-CD4	Dako/M0834	1:1000
Monoclonal anti-CD8	Dako/M7103	1:20
Monoclonal anti-CD20	Dako/M755	1:400
Monoclonal anti-CD57	Neomarkers/MS-136-P	1:200
Monoclonal anti-CD68	Dako/M814	1:200
Polyclonal anti-S100	Dako/Z311	1:1000
Monoclonal anti-Foxp3	Ebioscience/14-4776	1:50
Monoclonal anti-CD123	Ebioscience/14-1239-82	1:50
Polyclonal anti-IL4	RD systems/AF204NA	1:40
Polyclonal anti-IL6	RD systems/AF206NA	1:20
Polyclonal anti-IL10	RD systems/AF217NA	1:40
Polyclonal anti-IL13	RD systems/AF213NA	1:500
Polyclonal anti-IL17	RD systems/AF317NA	1:500
Polyclonal anti-IL18	Santa cruz/SC 6178	1:100
Polyclonal anti-TNF alpha	RD systems/AF210NA	1:20
Monoclonal anti-IFN-gamma	RD systems/MAB285	1:30

2. Materials and methods

2.1. Samples

Twenty formalin-fixed paraffin-embedded kidney specimens of fatal dengue cases were selected from the archives of the Department of Pathology of Hospital Guilherme Álvaro, municipality of Santos, São Paulo State, Brazil. Specimens were obtained during viscerotomy of human patients who died with DHF during the year of 2010, following the ethical standards of the institutional review committee. The patients' ages ranged from 4 to 62 years old, nine were male and 11 female.

Diagnosis was confirmed by correlation of clinical data, serologic diagnosis (IgM antibodies and NS1 antigens detection by ELISA) and histological examination by hematoxylin-eosin staining and detection of viral antigens through immunohistochemical assay [28,25]. The control group ($n = 5$) included fprmalin-fixed paraffin-embedded kidney specimens from patients who died without any infectious disease or renal damage, confirmed by histological evaluation.

2.2. Immunohistochemistry analysis

Sections were made from formalin-fixed, paraffin-embedded kidney samples and re-hydrated by a series of increasing ethanol gradient. Endogenous peroxidase activity was blocked by incubating sections in 3% H_2O_2 solution at room temperature for 20 min. For antigen retrieval, sections were incubated in retrieval solution (Dako, S2367) pH 9.0 for 20 min at 95 °C. Immunohistochemical staining was performed by incubation with specific primary antibody (Table 1). The tissue samples were incubated with the specific secondary antibody and a streptavidin-biotin peroxidase system, according to the manufacturer's instructions (LSAB system, code K0690, DakoCytomation, Carpinteria, CA, USA). Each reaction was visualized with 3,3′-diaminobenzidine-tetrahydrochloride (DAB) (Sigma–Aldrich Chemical Co., St. Louis, MO, USA D5637). All