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Proteinuria during dengue fever in children

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ABSTRACT

Objectives: This study aimed to investigate proteinuria occurring during dengue disease in children and assess if measurement of this parameter can help physicians in the clinical management of patients.
Methods: Proteinuria was assessed by dipstick and quantified by urine protein:creatinine ratio (UPCR) in samples from patients hospitalized with a confirmed dengue infection and in healthy controls.
Results: The dipstick tested positive in 42.9% of the patients presenting at hospital with dengue versus 20.0% in healthy controls. UPCR increased during the critical phase of the disease; peaking one week after fever onset then decreasing as the patients recovered. Patients with warnings signs or severe dengue were more likely to present with proteinuria detected by UPCR at the time of hospital admission compared to patients without warning signs. The sensitivity of this marker, however, was limited as only 16.1% of the patients with warning signs had proteinuria.
Conclusions: Urine dipstick and UPCR do not seem to be very valuable for the triage of the patients at the time of the initial consultation but the observation of a decrease of the UPCR during the course of the illness appears to indicate an evolution towards recovery.

Background

Dengue is the most prevalent mosquito-borne viral disease worldwide.[1] A majority of the infections are asymptomatic or result in a mild febrile illness, but the dengue virus (DENV) is also capable of producing a life-threatening disease. The main form of severe dengue is characterized by plasma leakage with or without bleeding, which may lead to circulatory collapse, called dengue shock syndrome. The course of dengue illness can be divided into three main phases: the febrile phase, the critical phase and the recovery phase. Severe clinical disease manifestations occur during the critical phase which begins around day 4–7 after the onset of fever and lasts usually 48–72 hours. During the critical phase, the condition of patients can improve or worsen rapidly; requiring careful monitoring by care givers. Early clinical management based on fluid replacement therapy reduces the morbidity and mortality associated with severe dengue.[2]

The major obstacle for an effective clinical management of dengue is the inability to accurately predict, at an early stage of infection, which patients are likely to develop a severe form of the disease. There is a need for simple, effective and cheap tests to identify patients at risk and guide triage. Wills et al. observed an increase of urinary protein clearance due to the increase in systemic vascular permeability that occurs in severe dengue. Subsequently, it has been proposed that a simple urine protein excretion screening test could be indicative of the severe form of dengue and therefore guide the triage and monitoring of the patients with suspected dengue infection.[3]

The objective of this study was to investigate the presence of proteinuria during dengue disease in children by simple urinalysis strip and by protein:creatinine ratio (UPCR) and assess if these parameters can help the physicians to improve the clinical

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management of dengue cases. To better characterize the dengue −associated proteinuria profiles, a urinary protein electrophoresis was performed in a subset of the hospitalized patients.

Methods

Clinical samples

A total of 241 spot urine samples obtained from 108 patients with laboratory-confirmed dengue virus infection (DVI) were randomly selected among samples obtained in 2013 during the DENgue research Framework for Resisting Epidemics in Europe (DENFREE) study. A confirmed dengue case was defined by the detection of viral RNA by RT-PCR and/or the detection of the NS1 protein and/or an IgM seroconversion and/or a four-fold antibody titer increase measured by hemagglutination inhibition assay (HIA) in paired plasma of patients presenting with symptoms suggestive of DVI. Most urine samples (225/241) were collected during hospitalization 1–11 days after the onset of fever. An additional 16 samples were collected one or three months after discharge from hospital. Patients were categorized as experiencing dengue "with" or "without" warning signs (WS+ or WS-, respectively) or severe dengue based on the 2009 case definitions established by the World Health Organization (WHO).[2] The immune status of patients (primary or secondary infection) was determined by HIA according to WHO criteria.[4]

A further 15 samples collected from healthy children recruited during a community-based study were also added to the panel. These controls were household members of some of the patients identified during the DENFREE hospital-based study that had no biological evidence of DVI (RT-PCR negative, NS1 negative, no anti-DENV IgM or increasing IgG titer).

Ethical statement

The DENFREE project was approved by the Cambodian National Ethics Committee for Health Research (authorization no. 063NECHR). The children's legal representatives signed a written consent before the enrolment of the patient.

Urine analysis

The presence of protein in urine collected at the time of patient admission at hospital was checked using semi-quantitative Mission urine dipsticks (Acon, San Diego, USA). The test was performed according to the manufacturer's instructions. Results were graded as: no protein; 150 mg/L (trace); 300 mg/L; 1000 mg/L; 3000 mg/L; and 20,000 mg/L. Presence of protein at any concentration was considered as a positive result. UPCRs were measured in samples collected during the course of hospitalization using a Cobas integra 400 plus analyzer (Roche Diagnostics, Germany). Proteinuria was defined as a UPCR $\geq$45 mg/mmol.[5]

Urine protein electrophoresis (UPEP) was performed with a MINICAP capillary electrophoresis platform (Sebia, France). Following the manufacturer's instructions, only samples with a total protein concentration >100 mg/L were tested.

Statistical analysis

Statistical tests were performed using STATA version 11.0 (StataCorp, USA). The statistical differences between categorical groups were detected using the Fisher's exact test. The Kruskal-Wallis rank test and the Mann Whitney U test were used for continuous independent variables and the Wilcoxon test was used for continuous dependant variables. The correlation between two continuous variables was assessed by Spearman's rank correlation test.

A Generalized Additive Mixed Model (GAMM) was used to evaluate factors independently associated with the UPCR values and with the occurrence of proteinuria. Five explanatory variables were included in the model: age, gender, sampling day after onset of fever (daof), immune status (primary or secondary infection) and 2009 WHO disease classification (dengue without warning signs, dengue with warning signs, severe dengue). A GAMM was used because a non-linear relationship between the response variables (UPCR or proteinuria occurrence) and both "daof" and "age" explanatory variables was expected. A mixed model was used with patient ID as a random effect explanatory variable to take into account the non-independence of samples collected from the same patients and potential random individual variations between patients. The analysis was performed using the "gamm4" package (version 0.2-3) under the R statistical environment (R Foundation, Vienna, Austria).

Significance was assigned at $p < 0.050$.

Results

A total of 108 patients were included in this study. A summary of the patients' characteristics, clinical and virologic data is presented in Table 1.

Protein detection by dipstick in urine specimens collected at admission

A total of 39 patients (42.9%) tested positive for proteinuria by dipstick at the time of admission to the hospital (Table 2). Most positive samples (71.8%, 28/39) only contained traces of protein (150 mg/L) while nine and two patients had an approximate protein concentration of 300 mg/L and 1000 mg/L, respectively. A positive dipstick result was observed for 38.1% (8/21) and 32% (16/50) of the patients admitted at hospital for a dengue WS- and WS+, respectively (Fisher's exact $P = 0.784$). Proteins were detected in the urine sample of 75% (15/20) of the patients presenting with a severe dengue. Three urine samples of the control group (20%, 3/15) contained traces of protein. The prevalence of proteinuria in dengue patients WS- and WS+ was not significantly different from the one obtained for the control group ($P = 0.295$ and 0.522, respectively). When considering the immune status of the patients, 53.2% (25/47) of those with a secondary infection and 26.9% (7/26) of the patients with a primary infection had proteinuria ($P = 0.048$).

Proteinuria determined by the UPCR during the course of hospitalization

The UPCR was tested at admission in 52 patients (median daof = 4, IQR = [3–4]) and proteinuria was detected in 23.1% (n = 12) of the cases. None of the WS- dengue cases had proteinuria compared with 16.1% (5/31) in WS+ cases and 50% (7/14) in patients experiencing severe dengue (Table 3). The prevalence of proteinuria at admission was not significantly different between primary and secondary dengue infections (13.6% vs 28.6%, $P = 0.281$) (Table 3).

The UPCR was measured in 178 urine samples collected during the course of hospitalization from nine WS- patients, 43 WS+ patients and 17 patients with a severe dengue. Nine samples were excluded from the analysis due to very low creatinine concentration (<1 mmol/L) that resulted in an overestimation of the UPCR. Proteinuria was observed in 33.3% and 23.3% of the WS- and WS+ patients, respectively, and 47.1% of the patients with severe dengue, (Table 3). Differences between the three groups of patients were not statistically significant. A total of 26.1% of the primary

Table 1
Summary of demographic, clinical and virologic information of the patients.

	Dengue without warning signs	Dengue with warning signs	Severe dengue
Number of patients (number of samples)	24 (45)	62 (143)	22 (53)
Median age in years [iqr]	7 [6–12]	8.5 [7–12]	9 [7–11]
Sex (Male)	15 (62.5%)	37 (59.7%)	15 (68.2%)
Median day of fever at hospital admission [iqr]	2 [2–3]	4 [3–4]	4 [4–5]
Median length of stay at hospital [iqr]	5 [5–5.5]	5 [4–6]	5 [4–5]
Dengue diagnostic			
RT-PCR and NS1 positive	19 (79.2%)	46 (74.2%)	9 (40.9%)
RT-PCR positive and NS1 negative	5 (20.8%)	11 (17.7%)	8 (36.4%)
RT-PCR negative and NS1 positive	0 (0%)	3 (4.8%)	1 (4.5%)
Other[a]	0 (0%)	2 (3.2%)	4 (18.2%)
Immune status			
Primary infection	10 (41.7%)	20 (32.2%)	0 (0%)
Secondary infection	11 (45.8%)	32 (51.6%)	21 (95.5%)
Undetermined[b]	3 (12.5%)	10 (16.1%)	1 (4.5%)
Clinical manifestations at admission			
Dry bleeding[c]	4 (16.7%)	15 (24.2%)	6 (27.3%)
Wet bleeding[d]	1 (4.2%)	10 (16.1%)	7 (31.8%)
Abdominal pain	0 (0%)	52 (83.9%)	22 (100%)
Persistent vomiting	0 (0%)	39 (62.9%)	22 (100%)
Lethargy/restlessness	1 (4.2%)	19 (30.6%)	15 (68.2%)
Hepatomegaly	0 (0%)	29 (46.8%)	15 (68.2%)
Ascites	0 (0%)	15 (24.2%)	8 (36.4%)
Pleural effusion	0 (0%)	12 (19.4%)	6 (27.3%)
Facial edema	0 (0%)	2 (3.2%)	3 (13.6%)
Cold extremities	0 (0%)	1 (1.6%)	19 (86.4%)
Cyanosis	0 (0%)	0 (0%)	11 (50%)
Hypotension, weak or undetectable pulse	0 (0%)	0 (0%)	12 (50.5%)
Narrow pulse pressure	0 (0%)	0 (0%)	16 (72.7%)
Delayed capillary refill time	0 (0%)	0 (0%)	14 (63.6%)

iqr: interquartile range.
[a] IgM seroconversion in paired plasma and/or fourfold increase of the antibody titer measured by hemagglutination inhibition assay (HIA) in paired plasma.
[b] Immune status is undetermined when the interval between admission and hospital discharge is <7 days and the HI titer at the time of hospital discharge is ≤2560.
[c] Petechiae, purpura and/or ecchymosis.
[d] Nose, gums bleeding, hematemasis, melena and/or conjunctival hemorrhage.

dengue cases and 33.3% of the secondary cases experienced at least one episode of proteinuria during hospitalization ($P = 0.768$). Proteinuria was more often detected between day 5 and day 7 after onset of fever when UPCR values were the highest (Figure 1). None of the controls had a UPCR ≥45 mg/mmol.

Sixteen patients included in the study were tested for UPCR one (n = 4) or three (n = 12) months after discharge from hospital. None had a UPCR ≥45 mg/mmol.

UPCRs according to the day of sampling after the onset of fever are shown in Figure 1. No significant difference was observed in UPCRs between the different groups of patients. UPCRs were significantly higher in each category of dengue patients at each time-point compared to controls.

Modeling factors independently associated with UPCR values in dengue cases

The "daof" variable and the age of the patient had a significant effect on UPCRs ($P < 0.001$) (Table 4). The GAMM-estimated relationships between UPCR and both "age" and "daof" variables are shown in Figure 2. The model predicted higher UPCR in urine samples collected from older children (≥13 years) and between DAOF 6–8. The immune status of the patient had no effect on UPCR values. A binomial GAMM was also used to assess factors independently associated with the occurrence of proteinuria in dengue patients, using the same variables. No significant association was found (Supplementary file 2).

Table 2
Results of urinary protein dipstick test performed at the time of admission to hospital.

	Positive dipstick, total	Positive dipstick, 150 mg/L (trace)	Positive dipstick, 300 mg/L	Positive dipstick, 1000 mg/L
Control (n = 15)	3 (20.0%)	3 (20.0%)	0 (0.0%)	0 (0.0%)
Total dengue (n = 91)	39 (42.9%)	28 (30.8%)	9 (9.9%)	2 (2.2%)
2009 WHO dengue classification				
Dengue without warning signs (n = 21)	8 (38.1%)	6 (28.6%)	2 (9.5%)	0 (0.0%)
Dengue with warning signs (n = 50)	16 (32.0%)	12 (24.0%)	4 (8.0%)	0 (0.0%)
Severe dengue (n = 20)	15 (75.0%)	10 (50.0%)	3 (15.0%)	2 (10.0%)
Immune status				
Primary infection (n = 26)	7 (26.9%)	7 (26.9%)	0 (0.0%)	0 (0.0%)
Secondary infection (n = 47)	25 (53.2%)	16 (34.0%)	7 (14.9%)	2 (4.3%)
Undetermined[a] (n = 18)	7 (38.9%)	5 (27.8%)	2 (11.1%)	0 (0.0%)

[a] Immune status is undetermined when the interval between admission and hospital discharge is <7 days and the HI titer at the time of hospital discharge is ≤2560.

Table 3
UPCRs in samples collected at the time of admission and during the course of hospitalization.

	Total dengue	2009 WHO disease classification			Immune status		
		Dengue without warning signs	Dengue with warning signs	Severe dengue	Primary infection	Secondary infection	Undetermined[a]
Hospital admission							
Number of patients tested	52	7	31	14	22	21	9
Number of patients with an UPCR ≥ 45 mg/mmol	12 (23.1%)	0 (0.0%)	5 (16.1%)	7 (50.0%)	3 (13.6%)	6 (28.6%)	3 (33.3%)
All samples (admission and during hospitalization)							
Number of patients included	69	9	43	17	23	33	13
Number of patients with an UPCR ≥ 45 mg/mmol in at least one sample	21 (30.4%)	3 (33.3%)	10 (23.3%)	8 (47.1%)	6 (26.1%)	11 (33.3%)	4 (30.8%)
Median UPCR (mg/mmol) [irq]	30.0 [22.1-42.2]	30.8 [20.5-43.0]	28.5 [21.6-38.5]	34.3 [25.7-48.8]	29.6 [22.1-43.3]	29.3 [21.6-48.8]	33.1 [23.6-40.9]
Median day of illness when UPCR ≥ 45 mg/mmol [irq]	6 [5–7]	6 [5–6.5]	6 [4–7]	6 [5–7]	5.5 [4–6.5]	6 [4–8]	6 [5–7]

UPCR: Urine protein:creatinine ratio.
iqr: interquartile range.
[a] Immune status is undetermined when the interval between admission and hospital discharge is <7 days and the HI titer at the time of hospital discharge is ≤2560.

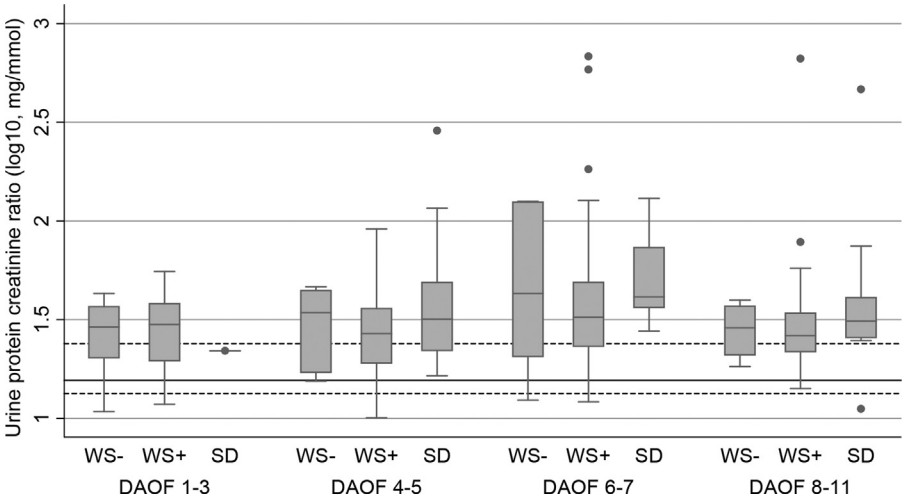

Figure 1. UPCR according to time of sampling and disease severity.
Horizontal solid lines represent the median value measured in control patients. Horizontal dash lines represent the quartiles. UPCR: Urine protein:creatinine ratio, DAOF: Day After Onset of Fever, WS-: dengue without Warning signs, WS+: dengue with Warning signs, SD: Severe dengue

Table 4
Effects of age, gender, disease classification, immune status and day of disease on UPCR.

Linear terms	**Estimate**	**p-value**
Male	reference	
Female	0.141	0.454
dengue WS-	reference	
dengue WS+	0.146	0.604
severe dengue	0.269	0.417
primary dengue	reference	
secondary dengue	−0.029	0.899
Non linear terms	**edf**	**p-value**
s(daof)	4.921	< 0.001
s(age)	3.414	< 0.001

edf: effective degrees of freedom of the smooth function terms (edf >1 indicate nonlinear relationships).
F value is an approximate F-test.
daof: day after onset of fever.

Association between serum total protein and UPCR

Serum total protein levels and UPCRs were assessed in parallel in 51 dengue patients. UPCRs tended to be higher in patients with hypoproteinemia (median = 31.6 mg/mmol, IQR = [22.1–48.9]) compared to patients with a normal serum protein concentration (median = 25.9 mg/mmol, IQR = [18.3–32.1]) but the difference was not significant ($P = 0.096$). A total of 38.1% (8/21) of the patients with hypoproteinemia had a UPCR ≥45 mg/mmol, whereas 10% (3/30) of the patients with a normal total protein concentration presented such a significant proteinuria ($P = 0.035$).

Urine protein electrophoresis

UPEP was performed on 27 admission samples in which the total protein concentration was ≥100 mg/L. Results are presented in Table 5.

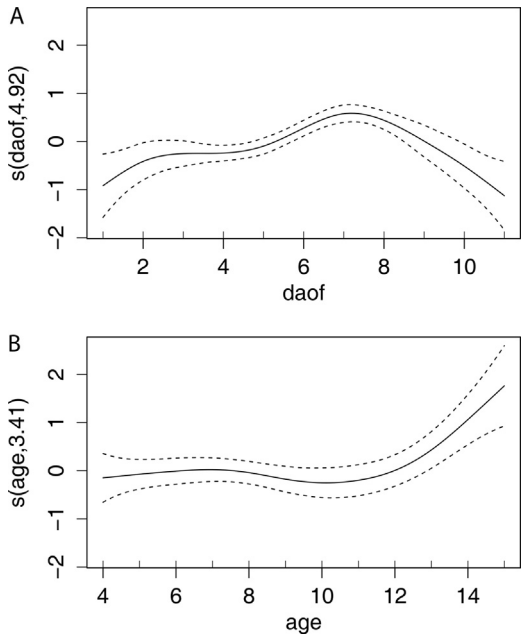

Figure 2. Relationship between UPCR and day of sampling after fever onset (A) or patient age (B).
A GAMM-model was used to estimate the relationship. The figure in the y-axis is the effective degrees of freedom of the smooth function terms. daof: day after onset of fever

Discussion

Increased urinary excretion of protein in patients infected by DENV is thought to be a hallmark of vascular endothelial cells defect and plasma leakage associated with complicated forms of dengue and is considered a possible prognostic marker.[3] Proteinuria has been reported in up to 74% of patients with dengue hemorrhagic fever (DHF)[6] and cases of self-limiting nephrotic-range proteinuria in patients with DHF presenting no manifestations of renal damage have been described elsewhere.[7,8] The mechanism underlying the hypothesis of increased protein excretion in urine is that during DVI the glycocalyx of the endothelial cells is disrupted either by direct action of the virus or by the NS1 antigen,[3,9,10] causing plasma leakage. At the kidney level, alteration of the glycocalyx layer that coats the glomerular endothelial cells enables the passage of macromolecules in the primary urine. In normal conditions, this passage is restricted in order to maintain the intravascular albumin concentration. As macromolecules cannot be reabsorbed by tubules, a glomerular proteinuria, mainly characterized by the presence of albumin in the final urine, occurs. Increased clearance of proteins in urine due to disruption of the glycocalyx has been described during diabetes mellitus,[11] childhood nephrotic syndrome[12] and meningococcal septicemia.[13]

We first investigated the presence of proteinuria by using a dipstick, one of the easiest and cheapest methods for the detection of proteins in urine. We observed that urinary dipstick used on samples collected at the time of initial medical consultation before admission to the hospital was not a reliable tool for the triage of dengue patients. The proportions of positive tests in controls (20%) and in dengue WS- (38.1%) and WS+ (32%) were not statistically different. Similar results were obtained when the patients were classified according to the 1997 WHO guidelines (data not shown). Vasanwala et al. and Lumpaopong et al. also assessed the prognostic value of urine dipstick and demonstrated that this rapid test was not able to discriminate between dengue fever (DF) and DHF.[14,15] In the present study, the comparison of the dipstick results with the UPCR results indicated that only 43.5% of the patients who tested positive for proteinuria by dipstick had a proteinuria considered as significant as defined by a UPCR $\geq$45 mg/mmol (data not shown). A proteinuria detected by dipstick and associated with a UPCR $<$45 mg/mmol was probably minor and/or the result of a urine concentration associated with a mild dehydration caused by fever or vomiting as previously reported.[16] The UPCR is actually a better tool to assess the proteinuria than simple dipstick as it intends to adjust for fluctuations in fluid intake and hydration status.

The gold standard to determine the proteinuria is provided by the measurement of total protein or albumin in urine over a 24-hour period (timed urine). However, this method is time-consuming and cumbersome. Measurement of urine albumin: creatinine or protein:creatinine ratios in random spot urine samples has been demonstrated to be an acceptable alternative to timed urine samples.[17,18] Since it was already demonstrated that urine albumin:creatinine ratios in dengue patients were not useful tools to predict the evolution towards severe dengue and the need for hospitalization,[19] we instead evaluated the potential value of the UPCR which was previously investigated in adults but not in children.[14] Using a more conservative definition of 45 mg/mmol as recommended by the Royal College of Physicians of London[5,20] we demonstrated that patients with WS+ and severe dengue were more likely to have an elevated UPCR at the time of hospital admission compared to WS- patients. Nevertheless, the sensitivity of this marker was limited as only 16.1% of the patients WS+ had such significant proteinuria.

Our multivariate analysis of UPCR during the course of the disease indicated that this ratio was significantly higher six to eight days after the onset of fever which is in agreement with previous reports.[14,19,21] Rather than being a marker that could be used for patient triage at the time of first medical consultation, UPCR measurement seems to be more useful for monitoring patients during the course of the disease. Indeed, the peak UPCR usually occurred at the end of the critical phase of the disease and the decrease in the ratio seemed to correspond with the beginning of the recovery phase.

Hypoproteinemia in dengue cases usually indicates a plasma leakage.[4] Here we observed that significant proteinuria was more

Table 5
Urine protein electrophoresis profiles in dengue patients.

Pattern	Number of observation				Mean UPCR
	Total (n = 27)	WS- (n = 2)	WS+ (n = 13)	SD (n = 12)	
Pattern 1: Normal	3 (11.1%)	0 (0.0%)	2 (15.3%)	1 (8.3%)	27.3 (min = 17.; max = 33.10)
Pattern 2: Alpha-1/Alpha-2	14 (51.9%)	2 (100%)	7 (53.8%)	5 (41.7%)	38.8 (min = 18.5; max = 73.5)
Pattern 3: Albumin	6 (22.2%)	0 (0.0%)	2 (15.3%)	4 (33.3%)	85.2 (min = 27.7; max = 130.2)
Pattern 4: "Serum-like"	4 (14.8%)	0 (0.0%)	2 (15.3%)	2 (16.7%)	298.12 (min = 114.5; max = 664.4)

UPCR: Urine protein:creatinine ratio.
WS-: Without warning signs; WS+: With warning signs; SD: Severe Dengue.

frequent in patients with hypoproteinemia but a UPCR ≥45 mg/mmol was observed in only 38.1% of the patients with hypoproteinemia. These results may suggest that the plasma leakage does not necessarily always cause significant proteinuria or that it is quickly compensated.

Three main categories of proteinuria can be distinguished by UPEP: overflow proteinuria, tubular proteinuria and glomerular proteinuria.[23] Overflow proteinuria is a pre-renal proteinuria characterized by an abnormal peak of one of the five protein fractions. UPEP in tubular proteinuria demonstrates alpha and/or beta globulin peaks, with or without a minor fraction of albumin. UPEP analysis of glomerular proteinuria shows either a dominant albumin fraction in selective glomerular proteinuria or a pattern similar to serum proteins in non-selective glomerular proteinuria. We did not observe any case of overflow proteinuria in the limited number of cases included in our study. Patterns compatible with a tubular proteinuria, a selective glomerular proteinuria and a non-selective glomerular proteinuria, were observed in 51.9%, 22.2% and 14.8% of the samples tested by UPEP, respectively. The glomerular proteinuria was associated with a high value of UPCR and we showed in this study that a UPCR ≥75 mg/mmol was 100% specific of a glomerular proteinuria. If we set a value of 75 mg/mmol for the UPCR as a hallmark of a glomerular proteinuria, 12 patients beyond the 21 that experienced a significant proteinuria could have been classified in that group. Among those 12 patients, one was classified as dengue without warning signs, six as dengue with warning signs and five as severe dengue. The hypothesis that the proteinuria in dengue disease was solely the result of disruption of the glycocalyx can only be partially true as we detected both tubular and glomerular proteinuria cases in almost the same proportions. Renal impairments associated to both glomerular and tubular syndromes have already been described.[6] Kidney biopsies from patients with dengue of varying degrees of severity revealed acute tubular necrosis resulting in tubular impairments.[24–28] Ischemic processes, which are common in patients with severe hypovolemic shock, have been suggested as an explanation to tubular necrosis in DSS patients.[25] Interstitial edema and mononuclear infiltration can also contribute to tubular necrosis in patients without shock syndrome.[24,27]

A limitation of our study was the small number of patients without warning signs as clinical specimens were only collected from hospitalized children and not from ambulatory cases. This resulted in an underrepresentation of the mildest form of dengue disease and may explain why we did not observe differences in proteinuria between patients with or without warning signs. Another limitation was the absence of daily UPCR measurement for each patient: the median number of samples tested for each patient was only two for a median length of hospitalization of five days. It is therefore possible that the occurrence of more significant proteinuria episodes were not captured in some patients. Another limitation of the study is that we were not able to explore potential confounding factors as number of other etiology of proteinuria do exist, including primary viral infections that are not uncommon in children (e.g., adenovirus, cytomegolavirus, Epstein-Barr virus, enterovirus, etc.).

The kidney is one of the major organs affected during dengue but the mechanism and the prevalence of renal involvement in children with dengue fever remains unclear. Renal function is not routinely assessed in patients hospitalized for suspected or confirmed dengue and thus the impact of dengue infection on the kidneys could well be under-recognized. This is a third limitation of our study as the proteinuria was measured retrospectively and the renal function was not assessed by the clinicians. There is a clear need, for a future study, to assess simultaneously the various parameters associated with a renal dysfunction as the hematuria, the proteinuria, an electrolyte disturbance, the glomerular filtration rate, and eventually to conduct renal biopsies. In conclusion, this study indicates that protein excretion in urine, estimated by UPCR, increases during the course of dengue disease to reach a maximum level approximately one week after the onset of fever. We found that neither urine dipstick nor UPCR measurement were useful for patient triage at the time of first visit to hospital. Monitoring the urinary protein excretion during the course of the disease, however, might be a factor indicating the evolution towards disease recovery.

Competing interests

Anne-Claire Andries is currently a Biosynex employee and Philippe Buchy a GSK vaccines employee.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijid.2016.12.022.

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