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Dengue Nonstructural Protein 1 Antigen in the Urine as a Rapid and Convenient Diagnostic Test during the Febrile Stage in Patients with Dengue Infection

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Abstract

A total of 136 matched serum and urine samples obtained from 55 patients with dengue infection and 30 other febrile illnesses were assayed for dengue nonstructural protein 1 (NS1) antigen. The urine NS1 ELISA was positive in patients with DF (68.4%) and dengue hemorrhagic fever DHF (63.9%), whereas the strip method showed a lower positive rate.

Dengue viral (DENV) infection can lead to broad range of clinical spectrum ranging from asymptomatic, undifferentiated fever, dengue fever (DF), and dengue hemorrhagic fever (DHF). Early diagnosis of DENV infection is important in patient management and control of dengue epidemic. To date, early detection of the virus is based on viral isolation or detection of the viral genome from serum samples. Recently, there were several case reports of detection of viral genome in saliva and urine by real time RT-PCR (Mizuno et al., 2007, Poloni et al, 2010). Detection of dengue viral antigen (DENV-NS1) is an alternative method that can be used for early diagnosis. It has several advantages over viral genome detection which cannot be performed in most laboratories. We hypothesized that DENV-NS1 may be detected in urine. The DENV-NS1 antigen strip is recently developed which can be used as a point-of-care rapid test with 98.9% sensitivity and 90.6% specificity compared to the enzyme immunoassay (Chaiyaratana et al, 2009). The DENV-NS1 strip to detect viral antigen in urine samples will be an ideal method for early detection of virus because of non-invasive, rapid, and do not require any equipment even centrifuge.

We report here the first result of detection of DENV-NS1 in urine samples and compare the result of DENV-NS1 strip to standard ELISA method. We recruited 85 patients aged 6 months to 18 years who were clinically suspected of having dengue infection admitted at Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital during March 2009 to July 2011. Ethical approval was obtained from the Faculty Ethics Committee and informed consent was obtained from parents. A total of 136 matched serum and urine samples were collected from 63 patients (1-5 samples per patients, with median 2). Time of sample collection ranged from 2 to 10 days after onset of fever. All patients presenting with warning signs required hospitalization. (WHO,2009). Severe dengue was diagnosed by severe plasma leakage leading to shock or fluid accumulation with respiratory distress.

Dengue viral infection is confirmed by viral isolation and/or presence of dengue specific IgM and IgG antibodies in acute and convalescent sera determined by capture ELISA. Primary DENV infection is defined when IgM/IgG ratio is ≥1.8.

Dengue NS1 antigen was determined in both serum and urine samples by enzyme immunoassay (Platelia Dengue NS1 Ag Kit, Biorad, Marnes-la-Coquette, France) and dengue NS1 antigen strip (Biorad, Marnes-la-Coquette, France) according the recommended protocol.

The results revealed 19 patients with DF and 36 patients developed DHF with evidence of plasma leakage (grade I, 14; grade II, 12; grade III, 7; grade IV, 3). All patients had serologic confirmation of DENV infection defined as primary infection (DF8, DHF9) and secondary infection (DF11, DHF27). Virus isolation revealed serotype 1 (DF2, DHF8), serotype 2 (DF2, DHF3), serotype 3 (DF2, DHF2) and serotype 4 (DHF1) in 20 patients while the remaining patients had negative results. Thirty patients were negative for evidence of acute DENV infection and were included as the controls.

The serum/urine NS1 detected by ELISA and strip are shown in Table 1. Positive rate of serum NS1 by ELISA was highest in DF (94.7%) but lower in DHF (66.7%). Comparing to ELISA, the strip method showed slightly lower positive rate in both DF (89.5%) and DHF (61.1%). We could detect NS1 in urine samples in both groups of patients. Positive rate of urine NS1 by ELISA in DF (68.4%) was slightly higher than that of DHF (63.9%). Comparing to ELISA, the strip method showed slightly lower positive rate in both DF (52.6%) and DHF (47.2%). Serum NS1 ELISA in primary DENV infection gave higher positive rate (88.2%), where 71.1% were detected in secondary infection. However positive rate in urine samples were similar in primary and secondary infection (70.6% and 63.2%, respectively). We could detect NS1 in urine sample but not in blood in only one DHF case. Thirty patients as the controls had negative DENV NS1 antigen for both serum and urine reflected the specificity of the test.

We report here the first evidence of DENV NS1 in urine samples from patients with DENV infection. We found a similar positive rate of NS1 in urine in patients with DHF and DF. Identification of DENV in urine has been reported (Poloni et al, 2010). We identified a higher positive rate of DENV NS1 in the urine samples. The positive rate was 95.8% when serum NS1 was positive in DHF but only 72.2% in DF. The higher positive rate of NS1in urine samples in DHF than DF and higher than the

positive rate of the virus itself suggested that detection of DENV NS1 in urine could be due to plasma leakage or kidney infection. Previous study demonstrated leaking of small size proteins such as albumin (MW, 59 kDa) and transferrin (MW, 79 kDa) and suggested impaired charge but retained size-dependent sieving mechanism of glomerular filtration (Wills et al, 2004). Dengue NS1 a 50-kDa non structural glycoprotein secreted as a soluble hexamer from DENV-infected cells. The secreted NS1 hexamer forms an open-barrel shape high density lipoprotein particle (Gutsche et al. 2011). The discrepancy in positive rate of NS1 in blood between DF and DHF and between primary and secondary infection has been reported (Chuansumrit et al, 2008). We tested positive rate of DENV NS1 strip technique comparing to ELISA as this technique can be used as a point-of-care diagnostic test. We confirmed our previous report of 98.9% sensitivity of the strip compare to ELISA method (Chaiyaratana et al, 2009). For urine, the strip method showed only 73.9 to 76.9% sensitivity compared to the ELISA method. There may be some technical problems such as pH, concentration etc that affect the sensitivity. Further studies are needed to improve the sensitivity of this method.

In conclusion, we demonstrated that DENV NS1 could be detected in urine samples during acute phase of DENV infection. The DENV NS1 strip technique could be ideal for this rapid diagnostic test but needs improvement. Whether NS1 in urine may reflect plasma leakage, and thus has prognostic value, needs further investigation.

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Table 1. Results of NS1 detection in serum and urine samples by ELISA and strip technique.

	Dengue fever (n=19)	**Dengue Hemorrhagic Fever (n=36)**
No.of positive serum NS1 by ELISA	18	24
sensitivity (%) (Wilson CI%)	94.7 (68.0-99.3)	66.7 (45.9-82.5)
specificity (%) (Wilson CI%)	100 (82.8-100)	100 (82.8-100)
PPV (%) (Wilson CI%)	100 (74.2-100)	100 (79.3-100)
NPV (%) (Wilson CI%)	96.8 (78.3-99.6)	71.4 (52.2-85.1)
No.of positive serum NS1 by strip	17	22
sensitivity (%) (Wilson CI%)	89.5 (61.6-97.8)	61.1 (40.6-78.3)
specificity (%) (Wilson CI%)	100 (82.8-100)	100 (82.8-100)
PPV (%) (Wilson CI%)	100 (73.1-100)	100 (77.9-100)
NPV (%)(Wilson CI%)	93.8 (74.5-98.7)	68.2 (49.3-82.5)
No.of positive urine NS1 by ELISA	13	23
sensitivity (%) (Wilson CI%)	68.4 (40.3-87.4)	63.9 (43.2-80.4)
specificity (%) (Wilson CI%)	100 (82.8-100)	100 (82.8-100)
PPV (%) (Wilson CI%)	100 (67.5-100)	100 (78.6-100)
NPV (%) (Wilson CI%)	83.3 (63.2-93.6)	69.8 (50.7-83.8)
No.of positive urine NS1 by strip	10	17
sensitivity (%) (Wilson CI%)	52.6 (27.1-76.8)	47.2 (28.4-66.8)
specificity (%) (Wilson CI%)	100 (82.8-100)	100 (82.8-100)
PPV (%) (Wilson CI%)	100 (61.5-100)	100 (73.1-100)
NPV (%) (Wilson CI%)	76.9 (57.1-89.3)	61.2 (43.5-76.4)