

## **APOPTOSIS OF PERIPHERAL BLOOD MONONUCLEAR CELLS IN CHILDREN WITH ACUTE DENGUE INFECTION**

**Myint KS, Endy TP, Mammen MP Jr, Kalayanarooj S, Vaughn DW, Nisalak A, Green S, Rothman AL, Ennis FA and Libraty DH**

Apoptosis is an important modulator of immune responses during systemic viral infections, but little is known about the role of apoptosis in dengue virus (DV) infections. Peripheral blood mononuclear cell (PBMC) apoptosis and plasma soluble levels of CD95 (sCD95), a mediator of apoptosis, were determined in coded samples from hospitalized children from 1995-1997. Apoptosis was examined in 58 children with dengue fever (DF), 59 children with dengue hemorrhagic fever (DHF) and 68 children with other febrile illnesses (OFI) by the TUNEL assay. Plasma sCD95 levels were determined by sandwich ELISA from 116 children. Around defervescence, PBMC apoptosis was higher in children with DHF, compared to DF ( $p=0.001$ ) and OFI ( $p<0.001$ ).  $CD8^+$  T-lymphocytes comprised at least half of the peak apoptotic PBMC in children with dengue as evidenced by dual color staining. Maximum plasma levels of sCD95 were also higher in DHF compared to DF ( $p\leq 0.03$ ). Apoptosis in PBMC is likely to be involved in modulation of the innate and adaptive immune responses to DV infection, and the degree of PBMC apoptosis correlates with dengue disease severity.

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## **CLADE EXTINCTION AND REPLACEMENT IN DENV-1 PHYLOGENIES IN THAILAND IS ASSOCIATED WITH CHANGING SEROTYPE PREVALENCE**

**Zhang C, Mammen MP Jr, Rodpradit P, Klungthong C, Monkongdee P, Chinnawirotpisan P and Holmes EC**

Virus envelop gene of 98 DEN-1 viruses and complete genome of 10 DEN-1 strains isolated from pediatric patients with varying degrees of clinical dengue severity in Queen Sirikit National Institutes of Child Health (QSNICH), Bangkok and Kamphaeng Phet Provincial Hospital (KPP) were sequenced to determine intra-serotype variations of DEN-1 viruses circulating in Thailand for the past 3 decades and the structure of viral genetic diversity in the locality and the evolutionary processes responsible for this structure; and investigate whether the E gene of DENV contains genetic information that correlates with disease severity. Our results showed that the DENV-1 strains sampled from Thailand fall into two of the three genotypes, vast majority of Thai DEN-1 strains fall into genotype I except five strains collected in 1980 and 1983 fell into genotype III. Within genotype I, the Thai strains fell into three distinct clades, two of are associated with different sampling times. Clear Phylogenetic groups associated with time of sampling are present in genotype I. Our data also showed that the branches separating the two clades of DENV-1 were defined by 13 amino acid changes. Of the 13, it was striking that four were located in the E gene, and most of these positions were invariant in the other serotypes suggesting that amino acid change at these positions will have major consequences for fitness. We propose that there is a

striking inverse correlation between the prevalence of DENV-1 and DENV-4 in the patients attending QSNICH in Bangkok, DENV-1 tends to peak in prevalence when DENV-4 is at low levels. More striking observation is that genetic diversity within DENV-1 peaks at times of high prevalence, and that clade extinction and replacement are associated with periods of low prevalence. No specific sequence pattern in E gene correlated with disease severity was observed.

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## DENGUE DIAGNOSIS IN NON-BLOOD SPECIMENS

**Kulwichit W, Mekmullica J, Prommalikit O, Suandork P, Krajiw S, Arunyingmongkol K, Pupaibool J, Jaimchariyatam N, Mutirangura A, Pancharoen C, Nisalak A and Thisyakorn U**

Dengue infection has become a worldwide problem in the past few decades, with worrisome figures of severe cases and mortality. Laboratory diagnosis relies on serologic and/or virologic tests performed on the patient's serum or plasma. We studied the feasibility of using urine and/or saliva in place of blood specimens. Samples of urine and saliva were obtained from patients admitted to the pediatric and adult wards in the late febrile or early convalescent period. Serologic results in serum/plasma were used as a gold standard. RT-nested PCR using primers targetting conserved sequences in the 5' untranslated region of the virus was done on urine specimens. MAC-ELISA detecting denguespecific IgG was performed in saliva and urine. Acute febrile patients with negative dengue serology in acute and convalescent sera served as negative controls.

Specimen type	Number tested	Test performed	# positive (%)	# +ve in controls (%)
Urine	48	RT-nestedPCR	40 (83.3)	0/18 (0)
Urine	22	ELISA	22 (100)	0/12 (0)
Saliva	31	ELISA	27 (87.1)	0/15 (0)

Our data indicate that RT-PCR and ELISA in urine and saliva are attractive candidates as dengue diagnostics in place of blood specimens. This would be highly applicable for pediatric cases who would unquestionably prefer 'noninvasive' specimens, or for epidemiologic studies in certain rural settings in developing countries, where blood drawing facilities might not always be available.

**1<sup>st</sup> Regional Meeting of Pediatric Dengue Vaccine Initiative (PDVI). Bangkok, Thailand. 18-20 October 2004.**