

ELEVATED SERUM ACID PHOSPHATASE LEVELS DURING DENGUE HEMORRHAGIC FEVER

Principal Investigators : Donald S. Burke, LTC, MC
Ananda Nisalak, M.D.
Suchitra Nimmannitya, M.D.

Associate Investigator : John Crum, MAJ, MSC

OBJECTIVE : To determine if the activity of a readily measured lysosomal enzyme, acid phosphatase (AP) is increased in the serum of children during the acute phase of dengue hemorrhagic fever (DHF).

BACKGROUND : There are two good reasons to suspect that serum levels of AP might be elevated during DHF.

1. Macrophages and reticuloendothelial cells, cell types rich in lysosomal enzymes (including AP) are probably the main site of replication of dengue viruses in vivo, and damage to those cells could lead to elevated serum levels of lysosomal enzymes and,

2. Huang (J. Kansai Med. Univ. 28: 5114-55, 1976) reported that in vitro cells infected with dengue viruses show a marked increase in intracellular AP activity.

We therefore attempted to measure AP activity in the sera of children with DHF and in the sera of appropriate control populations.

METHODS : Blood specimens were obtained in plastic syringes without anti-coagulant, transferred to glass tubes, and allowed to clot at room temperature for 2.4 hours. Serum was separated from the clot and kept at 4°C until assayed 1-2 hours later, always on the day of collection. Overtly hemolyzed samples were not analyzed. AP activity was measured by the method of Bessey (hydrolysis of nitrophenyl phosphate at pH 4.8) using commercial reagents (Sigma kit #104). In 10 patients with DHF, serum was obtained on the day of presentation and daily thereafter for 3 to 5 days until discharge, and a convalescent AP level was obtained 12-20 days after presentation.

RESULTS : The results are presented in tabular form. Tables 1 and 2 show that serum acid phosphatase levels in DHF patients are elevated compared to hospital and age matched febrile and normal control patients. Table 3 shows that elevated AP levels are not found in patients due to other causes (i.e., no seroconversion to dengue) even if a clinical diagnosis of DHF is made. Also, uncomplicated dengue fever without manifestations of DHF is not associated with elevated AP. Only in those cases in which the diagnosis of DHF was either firmly made or initially entertained, and which showed serologic evidence of dengue infection, is the mean serum AP level elevated. Between the grades of DHF there are no demonstrable differences in the AP levels. Table 4 shows that the acid phosphatase levels are not elevated in patients with thrombocytopenia who do not have both a clinical diagnosis of DHF and serologic evidence of dengue infection. In a similar analysis, among all 28 patients with DHF grade I, there was no

significant correlation between the platelet count and the serum AP level (mean AP = 2.11 ± 1.13 ; mean platelet count = $54.2 \pm 38.8 \times 10^3$).

$$\text{AP units/ml} = 2.361 - .005 \times (\text{platelet count} \times 10^{-3})$$

($r = -.161$, $p = \text{N.S.}$)

Table 5 shows the acute, mid-course, and convalescent serum AP levels in 10 patients; in 9 of the 10 cases the convalescent value is lower than all the acute values for that patient.

Although we could not demonstrate a statistical association of the AP level on admission and the grade of illness, the highest single value obtained was 5.55 in a 7 month old child who died of DHF.

Table 1. Serum acid phosphatase levels in normal Thai children, children with febrile illnesses other than dengue, and dengue hemorrhagic fever.

<u>Age</u>	<u>Normal</u>	<u>UF(Non-dengue)</u>	<u>DHF^a</u>
< 1	ND	1.36 ± .25 (11)	3.46 ± 1.04 (15)
1-2	ND	1.04 ± .23 (27)	2.20 ± .80 (9)
3-4	ND	1.10 ± .30 (28)	1.94 ± .64 (49)
5-6	1.34 ± .21 (10)	1.19 ± .39 (26)	1.94 ± .64 (62)
7-8	1.28 ± .26 (20)	1.07 ± .27 (20)	1.92 ± .77 (56)
9-10	1.13 ± .11 (10)	1.27 ± .34 (14)	2.23 ± .94 (34)
11-12	1.41 ± .32 (26)	1.22 ± .19 (8)	2.15 ± .98 (31)
13-14	ND	1.38 ± .26 (2)	2.33 ± .79 (19)
<hr/> Total	<hr/> 1.31 ± .28 (66)	<hr/> 1.15 ± .39 (136)	<hr/> 2.12 ± .86 (275)

^a For all age groups the mean for the DHF group is greater than the mean of the comparable normal and UF groups with a probability of $p < .005$

Table 2. Proportion of children with slightly and greatly elevated acid phosphatase levels.

	<u>Normal</u>	<u>UF</u>	<u>DHF</u>
Normal mean			
+ 1 S.D. (<u>></u> 1.60)	4/66(6%)	8/136(6%)	188/275(68%)
Normal mean			
+ 2 S.D. (<u>></u> 1.86)	4/66(6%)	3/136(2%)	150/275(55%)

Table 3. Serum acid phosphatase levels in normal children, children with non-dengue febrile illnesses, uncomplicated dengue fever, and DHF grades I through IV.

<u>Clinical Dx</u>		Serologic Diagnosis of acute dengue infection	N	Serum acid phosphatase level (Mean \pm 1 S.D.)
Initial	Final			
Normal	Normal	Not done	66	1.31 \pm 0.28
PUO	PUO	0	136	1.15 \pm 0.39
DHF	PUO	0	25	1.18 \pm 0.34
DHF	DHF	0	5	1.19 \pm 0.61
Measles	Measles	Not done	20	1.41 \pm 0.34
PUO	PUO	+	12	1.17 \pm 0.31
DHF	PUO	+	13	1.67 \pm 0.61
PUO	DHF	+	(3)	1.76 \pm 0.52
DHF	DHF Gr I	+	28	2.11 \pm 1.13
	Gr II	+	84	2.21 \pm 0.76
	Gr II-III	+	48	2.10 \pm 1.05
	Gr III	+	106	2.05 \pm 0.72
	Gr IV	+	9	1.87 \pm 0.70
	All (Gr I-IV)	+	275	2.12 \pm 0.86

Table 4. Serum acid phosphatase levels in children with undifferentiated fevers and dengue₃ fever with thrombocytopenia (platelets less than 100,000/mm³)

Clinical diagnosis		Serologic diagnosis of acute dengue infection	N	Platelet count (x10 ⁻³) (mean ± 1 S.D.)	Serum acid phosphatase level (mean ± 1 S.D.)
Initial	Final				
PUO	PUO	0	12	44 ± 23	1.15 ± 0.27
DHF	PUO	0	8	77 ± 14	1.07 ± 0.43
DHF	DHF	0	1	66	1.06
PUO	PUO	+	3	48 ± 33	1.22 ± 0.12

Table 5. Acute and convalescent acid phosphatase levels in patients with DHF

Case #	Day of illness							CONV
	3	4	5	6	7	8	9	
D80-442		1.82	1.23	1.10	1.15	1.16		1.00
-476		1.92	2.04	2.12				1.45
-533	1.24	1.60	1.41					1.58
-607			1.06	1.75	1.54			0.97
-687			1.15	1.04	1.34			0.92
-694*			2.60	2.72	2.45			1.41
-695			1.96	2.66	2.50			1.64
-697*		2.41	2.72	2.52	2.42			1.71
-719					2.15	2.10	2.49	1.05
-755			1.51	1.69	1.22			1.13
X	1.24	1.94	1.74	1.95	1.85	1.63	2.49	1.29
SD	-	0.34	0.62	0.67	0.59	-	-	0.30
N	1	4	9	8	8	2	1	10

* Patients with shock