Correlation between clinical and laboratory diagnosis of dengue in Sri Lanka

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INTRODUCTION

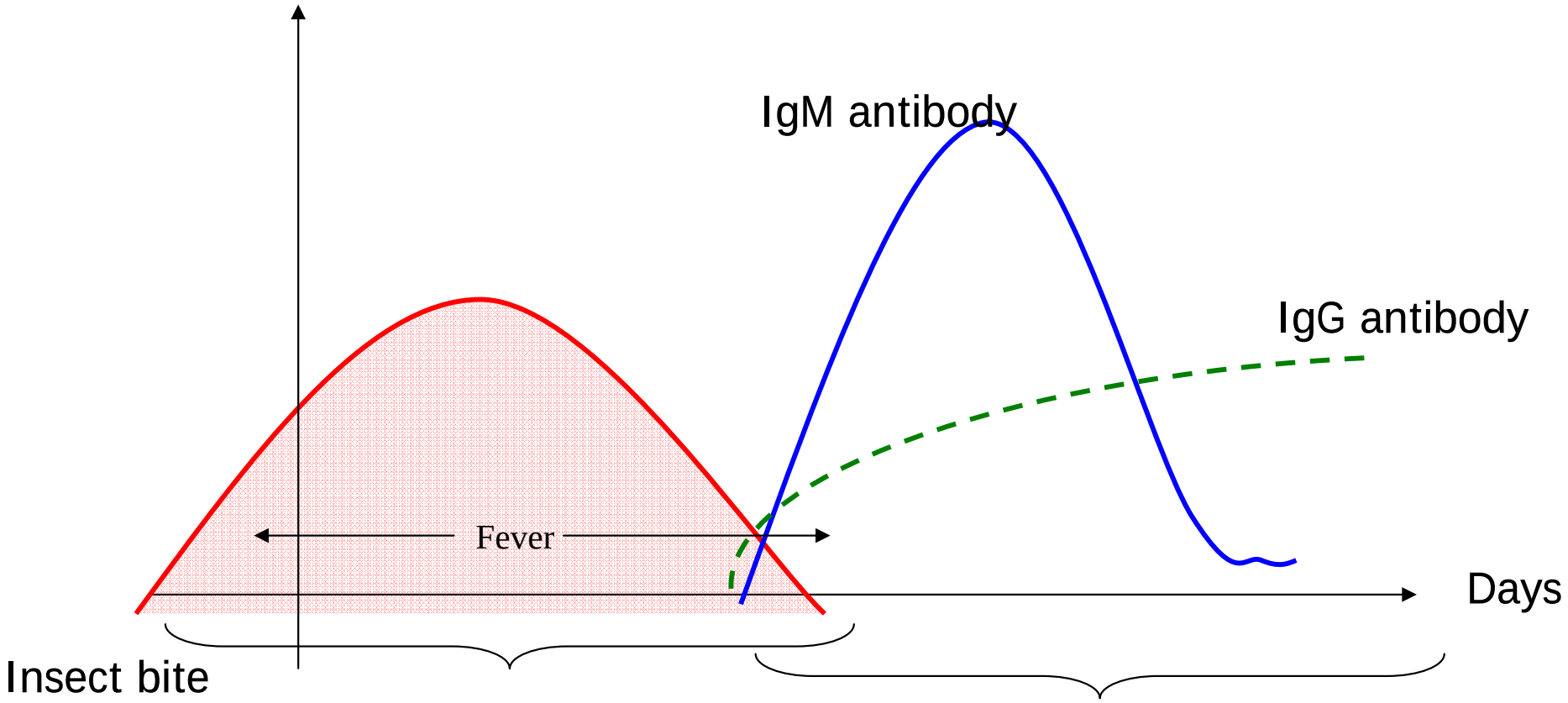
- **Dengue is an important vector-borne viral infection in South East Asia.**

- **Dengue virus is responsible for Dengue Fever (DF), Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS).**

- **In Sri Lanka diagnosis of dengue mainly depends on clinical signs and symptoms.**

- **Very few suspected patients are tested by laboratory diagnostic assays compared to the number of dengue cases recorded all over the island.**

Antibody response of dengue virus



Viremia

RT-PCR
Virus isolation
Antigen D. ELISA

Antibody response

ELISA
HAI
PRINT

OBJICTIVE

- **To analyze the actual number of definitive/probable dengue patients among dengue suspected patients in Sri Lanka using laboratory diagnostic assays.**

RESEARCH DESIGN

Human serum samples

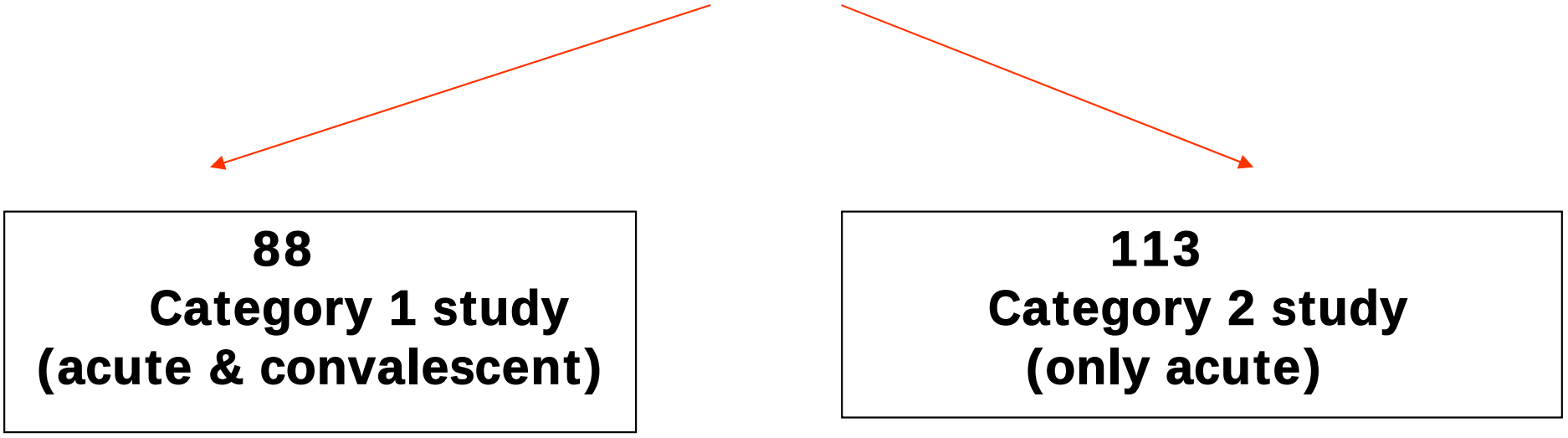
- **A panel of serum samples from 201 patients clinically suspected of having dengue warded at the North Colombo Teaching Hospital, Ragama, Sri Lanka were used.**

- **Five milliliters of venous blood was drawn from each patient over 18 years by a Medical Officer after obtaining informed written consent.**

- **Ethical permission for collection of human serum samples was obtained from the Ethical Review Committee, Faculty of Medicine, University of Kelaniya, Sri Lanka.**

- **Patients were categorized into 2 categories based on the availability of the convalescent serum samples.**

201 patients - clinically suspected of having dengue were selected based on WHO criteria



Clinical and laboratory information of selected patients

Interviewer administered questionnaire and the bed head ticket.

- **Fever days**
- **Highest fever recorded**
- **Age**
- **Sex**
- **Presence / absence of associated symptoms**
  headache, rectro-orbital pain, neck pain, limb pain
- **Bleeding manifestations & signs**
- **Platelet count**
- **Packed Cell Volume (PCV)**

Laboratory diagnosis of dengue

Research design cont.

Acute serum – (Category 1 and 2)

Molecular - RT-PCR-AGE (Chow *et al.*, 1993)
RT-PCR-LH (Gunesekera *at al.*, 2003)

Virological - Virus isolation (Chanyasanha *et al.*, 1995)

Serological - HAI assay (Clarke and Casals, 1958)
IgM capture ELISA (MRL diagnostics, USA)

Convalescent serum (Category 1 study)

Serological - HAI

Data processing and analysis

- **All data were recorded on forms designed for each purpose.**

- **Differences in clinical and laboratory data were analyzed on the basis of the final diagnosis assigned as confirmed dengue (totality of definitive and probable dengue cases), definitive dengue or non dengue (Horvath *et al*., 1999).**

Research design cont.

- **Definite cases required**
  viral detection by nucleic acid amplification/viral culture
  detection of 4 fold rise in anti-dengue IgG antibody in acute and convalescent serum samples by HAI assay

- **Probable**
  positive for IgM antibodies by ELISA

- **Non dengue**
  Negative by all laboratory assays

- **Analysis was carried out by comparing all dengue/definitive dengue infections with non dengue infections.**

- **Chi-squre test (Epi 6 Version 6.04d software, Centre for Disease Control, USA).**

- **Two variables were analyzed at a 95% confidence interval and P value <0.05 was considered as significant.**

RESULTS

Category 1 (n=88)

Dengue primary infection	**- 10**
Dengue secondary infection	**- 70**
Non dengue	**- 08**

Table 1. Laboratory diagnostic assays for acute serum samples collected from HAI assay confirmed dengue patients in Category 1 study (n=80)

Type of assay	No of patients positive (%) Fever ≤5 days (n=38)	No of patients positive (%) Fever >5 days (n=42)	No of patients positive (%) Total (n=80)
RT-PCR-LH	38 (100%)	25 (59%)	63 (79%)
RT-PCR-AGE	30 (79%)	00 (00%)	30 (37%)
Virus isolation	18 (47%)	00 (00%)	18 (22%)
IgM capture ELISA	00 (00%)	39 (93%)	39 (49%)

Table 2. Laboratory diagnostic assays for acute serum samples collected from HAI assay confirmed dengue patients in Category 2 study (n=113)

Type of assay	No of patients positive (%) Fever ≤5 days (n=41)	No of patients positive (%) Fever >5 days (n=72)	No of patients positive (%) Total (n=113)
RT-PCR-LH	19 (46%)	22 (30%)	41 (36%)
RT-PCR-AGE	15 (36%)	00 (00%)	15 (13%)
Virus isolation	12 (29%)	00 (00%)	12 (10%)
IgM capture ELISA	02 (07%)	51 (71%)	53 (46%)
HAI	09 (22%)	55 (76%)	64 (57%)

The proportion of laboratory diagnosed

dengue patients - 80% (162/201)
definitive dengue - 75% (121/162)
probable dengue - 25% (41/162)

Table 3. Results of laboratory confirmation of dengue suspected patients (n=201)

Type of category	No of definitive dengue patients (n=121)	No of probable dengue patients (n=41)	No of non dengue patients Total (n=39)
Category 1 (n=88)	**80**	**00**	**08**
Category 2 (n=113)	**41**	**41**	**31**
Total (n=201)	**121**	**41**	**39**

Results cont.

Table 4. Clinical presentation of selected patients

Clinical features	Dengue positive patients (n=162)	Definitive dengue patients (n=121)	Non dengue (n=39)
Associated symptoms			
Fever	162 (100%)	121 (100%)	39 (100%)
Headache	129 (80%)	104 (86%)	18 (46%)
Retro-orbital pain	52 (32%)	37 (30%)	10 (26%)
Neck pain	44 (27%)	29 (24%)	05 (13%)
Limb pain	107 (66%)	81 (67%)	18 (46%)
External bleeding	67 (41%)	58 (48%)	00 (00%)
Nasal	08 (05%)	07 (06%)	00 (00%)
Gum	08 (05%)	04 (33%)	00 (00%)
Haematemesis	02 (01%)	02 (02%)	00 (00%)
Melaena	05 (03%)	04 (03%)	00 (00%)
Skin patches	48 (30%)	41 (34%)	00 (00%)

Table 4. Correlation between clinical parameters and laboratory diagnostic assays

Clinical parameter	Dengue positive patients (n=162)	Definitive dengue patients (n=121)	
Platelet	92 247 mm^3 (20 000-3 18 000)	91 747 (20 000-318 000)	
PCV	45% (31-59)	45% (31-59)	

- **On comparison of the presence of clinical features that are used by the WHO for diagnosis of diagnosis of dengue,**

headache (129/162 vs 18/39, Chi=23, p=0.00)
limb pain (107/162 vs 18/39, Chi=5, p=0.00)
external bleeding (67/162 vs 00/39,Chi=27, p=0.00)

showed significant association with dengue.

DISCUSSION

- **Diagnosis of dengue infection based on clinical symptoms is not reliable and more than half of infected individuals either are asymptomatic or have a mild undifferentiated fever.**

- **The clinical presentation and blood counts were similar between patients hospitalized with acute dengue fever and patients with other febrile illness.**

- **Surveillance based on clinical diagnosis result in over estimation of the disease as clinical diagnosis is not specific enough.**

Results cont.

- **This over estimation of dengue cases was evident in this study.**

- **The proportion of laboratory diagnosed dengue patients were 80% (162/201) out of which 60% (121/201) were definitive dengue and 20% (41/201) were probable dengue.**

- **Laboratory confirmation of dengue suspected patients is important to measure the real incidence of the disease in Sri Lanka.**

- **This is important for effective treatment and reduce case fatality rate.**

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THANK YOU