DENGUE INFECTION;
STUDY FOR EVALUATION OF ENZYME IMMUNOASSAY (EIA) TEST FOR RAPID DIAGNOSIS

ORIGINAL
PROF-1806

DR. ATIF SITWAT HAYAT
MBBS, M.D (Medicine)
Consultant Physician
Assistant Professor Medicine
Isra University Hospital
Hala Road Hyderabad,
Sind, Pakistan.

DR. GHULAM HUSSAIN BALOCH
MBBS, Dip-Card, MD
Assistant Professor Medicine
Liaquat University of Medical and
Health Sciences Jamshoro, Sind, Pakistan.

DR. NAILA SHAIKH
MBBS, DCP
Senior Lecturer Pathology
Liaquat University of Medical and
Health Sciences Jamshoro, Sind, Pakistan.

ABSTRACT... Background: Dengue fever (DF) has emerged as a major public health problem across the world in terms of health cost, morbidity and mortality. **Objective:** The objective of our study was to determine frequency of seropositive dengue virus infection by using enzyme immunoassay (EIA) test in patients with probable dengue infection at a tertiary care hospital of Hyderabad. **Design:** This cross-sectional, observative , hospital-based study. **Setting:** Liaquat University Hospital Hyderabad. **Period:** 1st June 2010 to 31st December 2010. **Methods:** Patients presenting with acute febrile illness (i.e. documented fever of > 38˚C), skin rashes with or without bleeding manifestations (petechiae, epistaxis, hematemesis, menorrhagia or malena) plus cytopenias (leucopenia and / or thrombocytopenia) on peripheral smear examination, were evaluated for probable dengue virus infection. Serologic diagnosis has been carried out by using enzyme immunoassay (EIA) test with differential detection of IgM and IgG. **Results:** Out of 340 cases who fulfilled WHO criteria of probable dengue fever, 152(44.70%) were enzyme immunoassay (EIA) test reactive, while 188(55.29%) EIA non-reactive. The primary dengue infection was found in 102(67.10%) patients and secondary infection in 50(32.89%). Among both groups, males were predominantly affected. Majority of patients were hospitalized during the month of October. In addition, large number of patients aged between 13-35 years. Only two patients expired due to dengue shock syndrome and they were suffering from secondary dengue infection. Case fatality rate was 0.3% in our study. **Conclusions:** On conclusion, early detection of primary and secondary dengue virus infections via enzyme immunoassay (EIA) being important, as it is simple and rapid diagnostic tool having high sensitivity. This is especially valuable in alleviating psychological fear, disease-progression and mortality associated with dengue fever epidemics.

Key words: Dengue fever(DF), Dengue hemorrhagic fever (DHF), enzyme immunoassay (EIA) test, Aedes aegypti mosquito, primary dengue infection, endemic.

INTRODUCTION

Dengue fever has emerged as a major public health problem across the world in terms of health-cost, morbidity and mortality. It is endemic in more than one hundred countries in tropical and sub-tropical regions of the globe. According to World Health Organization (WHO)[1], two-fifths of world's population (i.e. 2500 million peoples) are now at risk for dengue, and annually approximately 50 million new cases of dengue fever (DF) occur worldwide with 500,000 cases of dengue hemorrhagic fever (DHF) requiring hospitalization every year and mortality rate of 2.5%. There is a dramatic increase for global prevalence of dengue fever in recent decades particularly in Americans, Western-Pacific and South-East-Asia[2]. Based on data from 112 national vital registration systems, 12,000 deaths in Southeast Asia, 4,000 in Western-Pacific and 2,000 in America for the year 2002, have been estimated due to dengue fever[3].

Dengue fever is a febrile illness caused by one of four antigenically different serotypes of dengue viruses (DEN-1, DEN-2, DEN-3 and DEN-4), which are the members of Flaviviridae family. It is mainly transmitted to humans via bite of Aedes aegypti mosquito[4]. Infection with a dengue virus may be clinically in-apparent or may be present as a non-specific febrile illness, classical dengue fever, dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). Although mild dengue disease and classical dengue fever contributes more than half of the total public health burden of dengue-associated illness[5], more serious manifestations of dengue hemorrhagic fever and dengue shock syndrome, provide major impetus for efforts to prevent infection[6]. In Pakistan, first outbreak of dengue fever was reported in

1994, followed by another epidemic in 2005[7]. Further outbreak occurred in upper parts of Punjab during 2003, in-addition to sporadic cases discovered at Rawalpindi-Islamabad, Peshawar, Jhelum, Abbottabad, Mangla and Haripur[8]. The largest outbreak has occurred in Karachi during 2006, causing maximum mortality[9], and this was associated with co-circulation of DEN-2 and 3 genotypes[10]. The enzyme immunoassay (EIA) test has demonstrated 100% sensitivity for rapid serological diagnosis of dengue fever showing results up to fifteen minutes, and able to distinguish between primary and secondary dengue virus infections through separate determinations of IgM and IgG antibodies.

This study was important in context of Hyderabad, where dengue fever outbreak has emerged especially after rains and floods. Furthermore, majority of patients have psychological fear of death from hemorrhagic complications of the disease. The objective of our study was to determine frequency of seropositive dengue virus infection by using rapid enzyme immunoassay (EIA) test in patients with probable dengue fever at a tertiary care hospital of Hyderabad. In dengue endemic areas like Hyderabad, rapid serological diagnosis is of paramount importance in order to plan preventive measures against dengue fever.

PATIENTS AND METHODS

This cross-sectional, observational, hospital-based study has been conducted at Liaquat University Hospital Hyderabad from 1st June 2010 to 31st December 2010. The local Ethical Committee of the Institute approved the study protocol and all patients gave written and informed consent.

Study Population

We approached all patients with 13 years or elder, being presented in medical wards of Liaquat University Hospital Hyderabad, with acute febrile illness (i.e. documented fever of > 38˚C) with or without bleeding manifestations (petechiae, epistaxis, menorrhagia, hematemesis or malena) plus cytopenias (leucopenia and or thrombocytopenia) on peripheral blood film examination. Patients were not eligible for the study if they had fever lasting longer than two weeks, an immunization in the preceding 48 hours, history of transfusion of blood or blood products (e.g. plasma or intravenous immunoglobulins) in the previous six months, hematological malignancies and bleeding disorders.

Patient were labelled as probable cases of dengue fever according to WHO criteria; acute febrile illness with two or more of the following manifestations; headache, retro-orbital pain, macular or maculopapular rash, myalgias, arthralgias, bleeding tendencies (like petechiae, epistaxis, menorrhagia, hematemesis or malena), leucopenia, thrombocytopenia and positive serology for dengue-specific IgM antibodies in venous blood samples drawn five or more days after onset of fever or occurrence at same location and time as other confirmed cases of dengue fever.

Sample Collection

Serum samples were drawn from all enrolled patients at the time of admission in medical wards. Acute phase sera were collected after five days of fever onset according to WHO criteria for serologic diagnosis of dengue fever using rapid enzyme immunoassay (EIA) test with differential detection of IgM and IgG antibodies. We have not taken convalescent phase sera, being taken 7-21 days after first sample.

Enzyme Immunoassay (EIA) Test

The enzyme immunoassay (EIA) test is a qualitative membrane based immunoassay for rapid detection of dengue-specific antibodies in whole blood or serum. This device consists of IgM and IgG components, being coated with ligand anti-human IgM and anti-human IgG antibodies respectively.

During this test, a drop of serum reacts with dengue antigen-coated particles (DEN-1 to DEN-4) in test strip made of nitrocellulose membrane. This mixture then migrates on the top of nitrocellulose membrane chromatographically via capillary action and being captured by lines of either anti-human IgM or IgG antibodies, hence forming a visible band at specific region. In-addition to anti-IgM and IgG lines, a central line was also included to ensure test validity. The results were graded as reactive (with visible band) and non-reactive (without band). In non-reactive cases, test has

been repeated after 3-5 days of first serum collection (i.e. within 12 days of fever onset).

The results of test were interpreted as; since IgG cut-off was set to detect secondary and not primary dengue infection, hence primary dengue virus infection was defined by a visible IgM band without visible IgG band, while secondary dengue infection was defined by a positive IgG band with or without positive IgM band. A negative result was defined by absence of both IgM and IgG bands (only control line visible). Other supportive laboratory investigations for dengue fever patients include; complete blood count (CBC), hematocrit, thick and thin blood film for material parasites, serum aspartate aminotransferase (AST), blood culture or typhidot test in selective cases. The bleeding, clotting and prothrombin times, platelet counts, urinary fibrin-degradation products (FDPs) and plasma fibrinogen levels in those with bleeding manifestations.

Management for study group

All patients with probable dengue virus infection were treated by adequate hydration in the form of oral and intravenous fluids. Acetaminophen (paracetamol4-6 tablets daily) has been prescribed for headache, fever, myalgias, arthralgias. Patients having dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) were immediately given intravenous fluids and blood transfusions. Platelet transfusions were given to patients with active bleeding and severe thrombocytopenia (platelet counts < 20,000 cells/mm^3).

Data Analysis

Data was recorded and analyzed by SPSS version 11.0. Relevant descriptive statistics such as mean, frequency and percentages were calculated for data presentation.

RESULTS

A total of 350 patients have been enrolled as suspected dengue fever, and 340 fulfilled WHO criteria of probable dengue virus infection. Amongst 340 cases, 152(44.70%) were enzyme immunoassay (EIA) test reactive, while 188(55.29%) EIA non-reactive.

Out of 152 EIA reactive cases, 116(76.31%) of patients have shown acute phase sera being positive after five days of fever onset, where as 36(23.68%) of patients demonstrated its positivity after 3-5 days (i.e. within 12 days of fever onset) later on repeated testing.

In EIA reactive group, primary dengue virus infection was found in 102(67.10%) patients and secondary infection in 50(32.89%). There were 101(66.44%) males and 51(33.55%) females with M to F ratio of 1.98:1. Majority of patients were hospitalized during the month of October 107(31.47%), followed by November 73(21.47%) and December 69(20.29%). This has been shown in table 1.

Table-I. Monthly Distribution of Patients with Probable Dengue Virus Infection (n=340)

Months	No. of patients	%age
June	08	2.35%
July	13	3.82%
August	23	6.76%
September	47	13.82%
October	107	31.47%
November	73	21.47%
December	69	20.29%

In-addition, large number 113(74.34%) have presented between ages 13-35 years. Amongst clinical features, all of our patients have presented with fever, followed by headache and myalgias. Abdominal pain was the least common symptom. Furthermore 31(20.39%) patients have been given platelet transfusions mainly due to active bleeding or thrombocytopenia. Out of 31 patients being transfused platelets, 24(77.41%) had secondary dengue infection while 7(22.58%) with primary dengue infection.

A total of 188(55.29%) patients with suspected dengue virus infection were found to be EIA non-reactive. Out of these, 128(68.08%) were males and 60(31.91%) females with M to F ratio of 2.13:1. Also in this group, large number 134(71.27%) had presented between ages 13-35 years.

In addition, 27(14.36%) patients had received platelet

Table-II. Clinical and Laboratory parameters of patients with probable dengue virus infection (n=340)

Parameters	EIA reactive (n=152)	EIA non-reactive (n=188)
Age (years)		
13-35	113 (74.34%)	134 (71.27%)
36-55	35 (23.02%)	45 (23.93%)
>55	04 (02.63%)	09 (4.78%)
Fever	152 (100%)	188 (100%)
Headache	115 (75.65%)	137 (72.87%)
Myalgias	102 (67.10%)	129 (68.61%)
Skin rashes	83 (54.60%)	101 (53.72%)
Arthralgias	63 (41.44%)	83 (44.14%)
Abdominal pain	41 (26.97%)	39 (20.74%)
Mucosal bleeding	37 (24.34%)	43 (22.87%)
Leucopenia (<4000 cells/mm^3)	39 (25.65%)	51 (27.12%)
Platelet counts		
(>100,000cells/mm^3)	73 (48.02%)	97 (51.59%)
(50,000-100,000cells/mm^3)	48 (31.57%)	63 (33.51%)
(<50,000cells/mm^3)	31 (20.39%)	28 (14.89%)

transfusions. Only two patients expired due to dengue shock syndrome and they were suffering from secondary dengue infection. Case fatality rate was 0.3% in our study. The details of clinical and laboratory parameters of both EIA reactive and non-reactive groups with probable dengue virus infection have been shown in table II.

DISCUSSION

Dengue is the most prevalent mosquito-borne viral disease. It is estimated that over 50 million dengue virus infections occur each year throughout the world[11]. Symptomatic dengue virus infections can present with a wide range of clinical manifestations, from a mild febrile illness to a life-threatening shock syndrome[12].

According to previous studies[9,13], maximum number of patients with dengue virus infection were hospitalized from August to November. This is quite similar to our study showing peak incidence of dengue fever from August to December and hence necessiating effective pesticide spray after rains and floods. The severity of dengue virus infection among different sexes has been reported in few hospital-based studies. Studies from Pakistan[13], Bangladesh[14] and India[15] have shown predominancy of males. The results of present study clearly demonstrated twice number of males than females in clinically proven dengue infections. The lowered rate of infections among females could be due to illiteracy and minimum exposure to this vector-borne disease. The presented data of our study has shown predominant involvement of young adults between 13-35 years by dengue virus infection. This is quite similar to local studies from Karachi[13,16] as well as studies from other endemic regions[17,18]. Similarly case-fatality rate was also more common among this age groups. This finding is consistent with a local study from Karachi[13]. The increased incidence and case-fatality rate of dengue fever among young adult males have focused for successful planning of public health programmes.

In our setup, diagnosis of dengue virus infection is primarily based on serology via detection of dengue-specific antibodies. Serological tests available for dengue fever include; hemagglutination inhibition (HI) assay, enzyme immunoassay (EIA) test and enzyme-linked immunosorbent assay (ELISA). The enzyme immunoassay (EIA) test is a commercial kit which incorporates four recombinant proteins from DEN 1 to DEN 4. It detects both IgM and IgG antibodies within fifteen minutes and thus help in differentiating between primary and secondary dengue infections.

During this study epidemic, we lacked the facility of specific diagnostic test for dengue virus isolation by cell culture and polymerase-chain reaction (RT-PCR) in our region. The dengue case detection were mainly based on IgM antibody only which was performed at the end of first week. No paired sera samples were selected, although IgM titers had been raised enough to be detected on seventh day. However, the possibility of missing few cases cannot be excluded.

RECOMMENDATIONS

- Public perception regarding dengue fever needs to be explored via electronic media.
- An effective disease prevention programme should include vector control by chemical, biological and/or environmental measures. The objective of such efforts is to decrease vector density so that future outbreaks of disease, leading to serious complications, can be minimized.
- Further comprehensive studies needed to identify dengue endemic areas in Pakistan, IgG sero-prevalence and sub-typing of virus in order to formulate effective preventive strategies and early detection.

CONCLUSIONS

Our study concludes, majority of patients with dengue virus infection were young, adult males, who presented with history of fever, Hence early detection of primary and secondary dengue virus infections via enzyme immunoassay (EIA) being important, as it is simple and rapid diagnostic tool having high sensitivity. This is especially valuable in alleviating psychological fear, disease- progression and mortality associated with dengue fever epidemics.

ACKNOWLEDGMENT

We are thankful to doctors and paramedical staff of Liaquat University Hospital Hyderabad for their co-operation in data collection. Also Mr. Zohaib Ahmed Khan has helped us in typing this manuscript. Conflict of interest none, study was conducted by utilizing the departmental resources.

REFERENCES

1. World Health Organization, WHO (2002) **Dengue and Dengue hemorrhagic fever.** Fact sheet no 117.WHO Geneva Available at :http://www.who.int/mediacentre/factsheets/f117/en/print.html.

2. Guha-sapir D, Schimmer B. **Dengue fever: new paradigms for a changing epidemiology.** Emerging themes in Epidemiology, 2005. Open access journal http://www.ete-online.com/content/2/1/1.

3. World Health Organization. World health report (2004). WHO, Geneva.

4. Guzman MG, Kouri G. **Dengue: an update.** Lancet Infect Dis 2001;2:33-42.

5. Anderson KB, Chunsuttiwat S, Nisalak A. **Burden of symptomatic dengue infection in children at primary school in Thailand: a prospective study.** Lancet 2007; 369:1452.

6. Halstead SB. **The XXth century dengue pandemic: Need for surveillance and research.** World Health Stat Q 1992; 45:292.

7. Jamil B, Hasan R, Zafar A, Bewley K, Chamberlain J, Mioulet V. **Dengue virus serotype 3, Karachi, Pakistan.** Emerg Infect Dis 2007; 13: 182-3.

8. Ali N, Nadeem A, Anwar M, Tariq WU, Chotani RA. **Dengue fever in malaria endemic areas.** J Coll Physician Surg Pak. 2006;16(5):340-2.

9. Khan E, Siddiqe J, Shakoor S, Mehraj V, Jamil B, Hassan R. **Dengue outbreak in Karachi Pakistan, 2006: experience at tertiary care center.** Trans R Soc Trop Med Hyg 2007;101:1114-9.

10. Khan E, Hassan R, Mehraj V, Nasir A, Siddiqe J, Hewson R. **Co-circulations of two genotypes of dengue virus in 2006 out-break of dengue hemorrhagic fever in Karachi, Pakistan.** J Clin Virol 2008;43:176-9.

11. Kroeger A, Nathan M, Hombach J. **Dengue.** Nat Rev Microbiol 2004; 2:360.

12. Teixeira MG, Barreto ML. **Diagnosis and management of dengue.** BMJ 2009;339:b4338.

13. Kidwai AA, Jamal Q, Saher, Mehrunnisa, Farooqi FR, Ullah S. **Serodiagnosis of dengue infection using rapid immunochromatography test in patients with probable dengue infection.** J Pak Med Associ Karachi. Nov 2010; 60(11): 936-939.

14. Ynus E. **National guidelines for clinical management of Dengue and Dengue Hemorrhagic Fever.** Dhaka, Disease Control Directorate, Directorate General of Health Services;2000.

15. Agarwal R, Kapoor S, Nagar R, Misra A, Tandon R, Mathur A, Misra AK, et al. **A clinical study of the patients with dengue hemorrhagic fever during the epidemic of 1996 at Lucknow, India.** Southeast Asian J Trop Med Public Health 1999;30:735-40.

16. Riaz MM, Hilal H, Mumtaz K, Khan MS, Patel J, Tariq M. **Outbreak of dengue fever in Karachi 2006:** a clinical perspective 2009;59:339.

17. Rahman M, Rahman K, Siddque AK, Shoma S, Kamal AH, Ali KS, Nisaluk A, et al. **First outbreak of dengue hemorrhagic Fever, Bangladesh.** Emerg Infect Dis 2002; 8: 738-40.

18. Low JGH, Ooi EE, Tolfvenstam T, Lew YS, Hibberd ML, Ng LC. **Early dengue infection and outcome study(EDEN) -study design preliminary findings.** Ann Acad Med Singapore 2006; 35: 783-9.

Article received on: 25/06/2011 Accepted for Publication: 15/08/2011 Received after proof reading: 02/12/2011

Correspondence Address:
Dr. Atif Sitwat hayat
Consultant Physician
Assistant Professor Medicine
Isra University Hospital
Hala Road Hyderabad, Sindh
ashayat@hotmail.com

Article Citation:
Hayat AS, Shaikh N, Baloch GH. Rapid diagnosis of dengue infection; Study for evaluation of enzyme immunoassay (EIA) test at Tertiary Care Hospital of Hyderabad. Professional Med J Dec 2011;18(4): 687-692.

PREVIOUS RELATED STUDIES

- Irfan Arshad, Fayyaz Ahmed Malik, Aamir Hussian, Shahida A.R. Shah. Dengue fever; clinico-pathologic correlations and their association with poor outcome. Prof Med Jour 18(1) 57-63 Jan, Feb, Mar 2011.

AN EXCELLENT MAN;
HE HAS NO ENEMIES;
AND NONE OF HIS FRIENDS
LIKE HIM.

OSCAR WILDE