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EVALUATION OF FOUR ELISA BASED IMMUNOASSAYS FOR THE DETECTION OF IgM ANTIBODIES AGAINST DENGUE VIRUS

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Introduction: *Dengue fever is a problem of serious concern which is mainly transmitted by mosquito, Aedes aegypti. These days' different ELISA kits are used for the diagnosis of dengue fever. It was a validation study. Study was conducted in the Department of Immunology at University of Health Sciences, Lahore during the period of August 2009 to October 2009.* ***Materials and Methods:*** *In this study four ELISA kits (Human, Nova Tech, Vircell and DRG) were used to determine IgM antibodies against dengue fever in forty four patients who were labelled positive for dengue fever by different commercially available ELISA kits.* ***Results:*** *Human ELISA kit gave most accurate results with respect to the agreement, sensitivity, specificity and κ value.* ***Conclusion:*** *Human ELISA kit was found most reliable for the diagnosis of dengue fever.*

Key words: *Dengue fever, Aedes aegypti, ELISA.*

INTRODUCTION

Dengue fever (DF) is a mosquito-borne infection which has become a major worldwide public health concern. DF is found in tropical regions around the world, predominantly in urban and peri-urban areas.[1] One of its lethal complications is dengue haemorrhagic fever (DHF) which was first recognized during 1950s and even today it is a leading cause of childhood deaths in many countries. Dengue fever virus is a flavivirus and is closely related to yellow fever virus, Japanese encephalitis virus and other group B Arboviruses.[2] There are four distinct viruses which may cause dengue infection but none of these offer protection against subsequent infection by other three types. The four serotypes DEN_1, DEN_2, DEN_3, and DEN_4 are transmitted by several mosquito species including *Aedes aegypti* and *Aedes albopictus*.[1]

Dengue fever is severe, flu-like illness that affects infants, young children and adults but rarely causes death. Infants and young children may have an undifferentiated febrile disease with rash. Older children and adults may have either mild febrile syndrome or classical incapacitating disease with abrupt onset and high grade fever, severe headache, pain behind the eyes, muscle and joint pains, and rash. Dengue hemorrhagic fever is a potentially deadly complication that is characterized by high grade fever, haemorrhagic phenomena and in severe cases, circulatory failure. The usually lethal dengue shock syndrome (DSS) may follow DHF after circulatory collapse.[3] Dengue viruses are transmitted to humans through the bite of infected *Aedes* mosquitoes. Once infected, mosquito remains infective for life, it transmits virus to susceptible individuals during probing and blood feeding. Infective female mosquitoes may also transmit virus to next generation of mosquitoes by transovarial transmission. Humans are main amplifying host of the virus and they circulate virus in their blood at approximately same time as they have fever, and other *Aedes* spp. mosquitoes may acquire the virus if they feed on an individual at this time.[4]

The global prevalence of DF has grown dramatically in recent decades. The disease is now endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, the Southeast Asia and the Western Pacific. About 2.5 billion people or two fifths of the world population are now at risk from dengue fever. WHO currently estimates, there may be 50 million cases of DF worldwide every year. Not only is the number of cases are increasing as the disease is spreading to new areas, but explosive outbreaks are occurring in various parts of the world. An estimated 500,000 cases of DHF require hospitalization each year.[5] The most challenging problem associated with patient management in dengue infection is rapid diagnosis. Early symptoms of DF mimic other diseases often prevalent in areas where DF is endemic, such as malaria, leptospirosis and even influenza. Thus a rapid differential diagnosis is crucial to proper patient care. The traditional diagnosis of dengue infection is performed by using hemagglutination inhibition (HAI)

assays, immunochromatogrphic, immunoblot assay or immunoglobulin M (IgM) capture enzyme linked immunosorbent assays (ELISA) on paired samples.[1,6]

Differences in assay formats, usage of antigens, and detection systems, make it difficult to estimate the value of each individual assay without prior comparison. This prompted us to evaluate four commercially available ELISA immunoassay systems for the detection of IgM antibodies to dengue virus in patients diagnosed with dengue infection.

MATERIAL AND METHODS

Forty four (44) positive samples for dengue fever by ELISA method were collected from laboratories of Children Hospital Lahore and Shalamar Hospital Lahore. The samples were collected at different times after the onset of clinical symptoms. It was a validation study. These patients had a history of fever with clinical suspicion of dengue fever. The level of anti-dengue IgM antibodies was determined using commercially available quantitative sandwich enzyme immuno-assay systems from Human (Human Gesellschaft for Biochemica and Diagnostica mbH Germany), Vircell (Vircell, Santa Fe Granda Spain), Nova Tec (Nova Tec Immunodiagnostica GmbH Germany), and DRG (DRG International Inc. USA).

The diagnostic kits were used and their results were interpreted according to manufacturers' directions. Measurements of absorbance were taken at wavelengths specified by kit's manufacture using automated ELISA plate reader (Mod # 680 Bio-Rad UK). Calculation of cut-off values for various systems was calculated according to instructions given in respective kits. Patients' samples having absorbance values of more than 10% of cut-off were considered positive while samples with absorbance values of less than 10% of cut-off were considered negative and samples of absorbance values in between ± 10% of cut-off range were considered borderline/ equivocal.

RESULTS

Among the 44 dengue positive samples tested by different ELISA kits, 37, 29, 27, and 24 samples were positive by Human, Vircell and Nova Tech kits respectively (Figure 1).

Figure 2 demonstrates the results of performance (sensitivities and specificities) of the four different immunoassays for detection of DEN IgM antibodies in serum samples from 44 patients with suspected acute DEN infection. The results of the individual assays with respect to agreement, sensitivity, specificity and κ value, using the consensus value as gold standard are summarized in Table 1, 2, 3 and 4. DRG kit was compared with other three kits for the detection of IgM antibodies against dengue virus and it was found that its sensitivity, specificity, diagnostic accuracy and agreement against Nova Tech was 88.89, 100.00, 93.18 and 86.08 respectively. Similarly, sensitivity, specificity, diagnostic accuracy and agreement of DRG kit against Vircell was 96.30, 82.35, 90.91 and 80.40 while against Human these values were 100.00, 41.18, 77.27 and 46.20 respectively (Table 1).

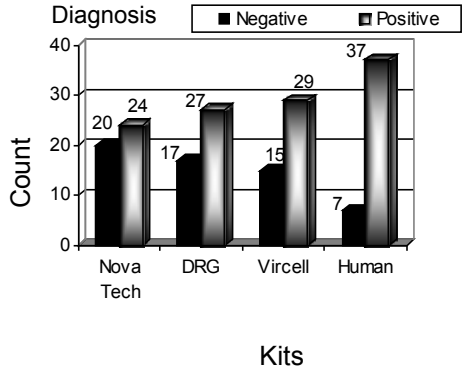

Fig. 1: *Characterization of patients according to different ELISA Kits for dengue virus.*

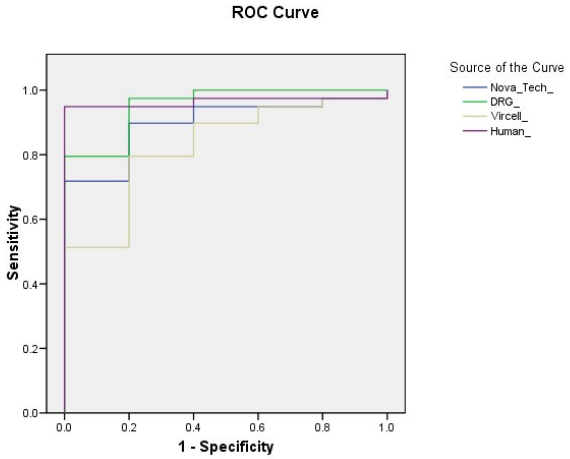

Fig. 2: *Sensitivities and specificities of various ELISA kits for the diagnosis of dengue fever.*

The sensitivity, specificity, diagnostic accuracy and agreement of Nova Tech against DRG, Vircell, and Human are summarized in Table 2. Table 3 and Table 4 explains the sensitivity, Specificity, diagnostic accuracy and agreement of Vircell and Human against other three kits.

Discussion

Keeping in view the complications and fatality rate associated with dengue fever, a rapid and accurate method for its diagnosis is important for proper

patient management. Dengue fever can be diagnosed by various methods like virus isolation, serology and reverse transcriptase PCR. All over the world most of the diagnostic centers use serological techniques for its detection. Detection of IgM antibodies is a sensitive method, but until recently, IgM assays were not widely available for use in many laboratories of world. Several studies have compared and evaluated different serological tests available for the diagnosis of dengue viral infection.[7-10]

We have evaluated four dengue ELISA immunoassays which were being used in various diagnostic laboratories of the town. Based on a consensus model, performance of each immunoassay was validated by using serum samples from patients with suspected DEN infections. In addition relative sensitivities of respective assays were studied with serum samples. In general all assays were easy to perform, but the simplest and fastest assay to perform was with Human ELISA kit. Detection of dengue IgM antibody seems to be a better option as it appears quite early in the course of ill-ness and its detection requires a single, properly timed blood sample and more over its testing procedure is relatively easy, compared to other classical methods. Further, IgM responses are usually less virus cross reactive.[11]

Depending upon the status of disease and laboratory facilities available, easier and reliable option for diagnosing dengue viral infections can lead to better patient management and effective control measures. Therefore it may prevent outbreaks and reduce associated case fatalities. Therefore, diagnosis of a particular flavivirus should always be made by considering clinical presentations of patients, performance characteristics of the serological test employed and knowledge of flaviviruses circulating in that particular geographical region. These commercial immunoassays have made serodiagnosis of DEN infection available to general laboratories.

Table 1: *Evaluation of DRG kit with other kits for detecting IgM antibodies against dengue Virus.*

	Sensitivity (%)	Specificity (%)	Diagnostic accuracy (%)	Agreement (%)
Nova-Tech	88.89	100.00	93.18	86.08
Vircell	96.30	82.35	90.91	80.40
Human	100.00	41.18	77.27	46.20

Table 2: *Evaluation of Nova Tech kit with other kits for detecting IgM antibodies against dengue virus.*

	Sensitivity (%)	Specificity (%)	Diagnostic accuracy (%)	Agreement (%)
DRG	100.00	85.00	93.18	86.08
Vircell	95.83	70.00	84.09	67.23
Human	100.00	35.00	70.45	37.00

Table 3: *Evaluation of Vircell kit with other kits for detecting IgM antibodies against dengue virus.*

	Sensitivity (%)	Specificity (%)	Diagnostic accuracy (%)	Agreement (%)
DRG	89.66	93.33	90.91	80.41
Nova Tech	79.31	93.33	84.09	67.23
Human	89.66	26.67	68.18	18.73

Table 4: *Evaluation of Human kit with other kits for detecting IgM antibodies against dengue virus.*

	Sensitivity (%)	Specificity (%)	Diagnostic accuracy (%)	Agreement (%)
DRG	72.97	100	77.27	46.21
Nova Tech	64.86	100	70.45	37.00
Vircell	70.27	57.14	68.18	18.73

It is **concludes** in the light of this study it is concluded that Human ELISA kit could be a good choice for the detection of IgM in dengue fever.

DISCLAMATION

It is strongly reported that authors have no financial or other interests in any of these commercially available ELISA kits.

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