Laboratory Diagnosis of Dengue: Utility of Combination of Diagnostic Tests at Tertiary Care Hospital

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Research Article

Abstract: **Introduction:** Early and accurate diagnosis of dengue is important for appropriate therapy. No single test can satisfactorily diagnose dengue at all stages of infection. Tertiary care centres face challenge of diagnosis of dengue using a single sample. **Aims:** To study diagnostic utility of RT –PCR, NS1 antigen detection along with IgM antibody detection tests using single serum sample per patient. **Settings and Design:** cross-sectional study. **Methods and Material:** Serum samples were collected from 181 patients clinically suspected of dengue infection at the time of admission. Samples were tested by using Panbio NS1 antigen ELISA, Real-time reverse transcriptase PCR (RT-PCR) and IgM ELISA. Statistical analysis used: Results were analyzed using Fisher's exact test **Results:** Of the 181 patients tested, 72 were positive by any of the three tests i.e. RT-PCR, NS1antigen ELISA or IgM ELISA. Considering PCR as gold standard, the sensitivity, specificity, positive predictive value and negative predictive value of NS1antigen ELISA was 46.15%, 93.51%, 78.26 % and 77.42% and that of combined usage of NS1antigen ELISA along with IgM ELISA was 71.79%, 84.42%, 70% and 85.53% respectively. The sensitivity of NS1antigen ELISA alone and in combination with IgM ELISA was significantly low in comparison with PCR. **Conclusions:** PCR is the preferred test to diagnose dengue infection in patients with acute stage of infection. In setups lacking PCR facility, NS1antigen ELISA can be used to confirm the diagnosis using single serum sample.

Keywords: Dengue, NS1antigen ELISA, RT-PCR, IgM ELISA

Introduction

Dengue is a major public health concern globally. World Health Organization estimates that 2.5 billion people are at risk in 100 tropical and subtropical regions of the world. Fifty million dengue infections occur worldwide annually. It affects up to 100 million people each year with 500,000 cases of DHF and DSS causing around 30000 deaths. [1, 2] Early diagnosis of disease helps in reducing the mortality significantly and will help in definitive management and will reduce the indiscriminate use of antimalarials and antimicrobials for treatment of patients with febrile illness. Dengue infection can be detected in the laboratory by isolation of the virus, demonstration of a rising titer of specific antibodies in paired serum samples, demonstration of a specific viral antigen (NS1) and demonstration of RNA in the tissue or serum.[3] Laboratory diagnosis of dengue differs depending upon the time of presentation of the patient to healthcare facility. Viral isolation remains the gold standard but requires high level of technical skills and infrastructure, hence cannot be used for routine diagnosis. RT-PCR which detects viral RNA is highly sensitive in early stage of infection and is a good tool, but remains expensive, requires technical expertise and infrastructure which limits its use especially in developing countries. Dengue NS1 antigen can be detected from first day and up to 14 days of infection.[4] IgM ELISAs can detect infection from fifth day onwards up to over a period of a month. The present study was carried out to determine the utility of NS1 antigen and IgM antibody detection for early diagnosis of suspected dengue infections and to compare it with RT- PCR using single serum sample.

Subjects and Methods

A cross sectional study was carried out over a period of six months on patients admitted with undifferentiated fever after institutional ethical committee approval. Out of total 2079 febrile patients, those having other laboratory confirmed infection (malaria, typhoid and leptospirosis) were excluded from the study. One hundred and eighty one patients strongly suspected of dengue were included in the study. From all the suspected dengue patients, 5ml of blood was collected. Serum was separated and stored in two aliquots. The first aliquot was used for detection of NS1 antigen (Pan –E DENGUE EARLY ELISA by Panbio; Australia) and dengue IgM antibodies (Nova Lisa by Nova Tec Immunodiagnostica GMBH, Dietzenbach, Germany). The second aliquot was sent to reference laboratory for dengue RT-PCR test (Cobas TaqMan Thermal cycler, Roche Applied

Sciences, Germany). The results of the two were blinded until final analysis.

Statistical Analysis

The data was analyzed and P value determined using Fisher's exact test. P value of <0.05 was considered significant. The sensitivity, specificity, positive predictive value and negative predictive values were calculated considering PCR as gold standard.

Table 1: Accuracy indices of NS1 antigen ELISA alone and in combination with IgM ELISA in comparison with PCR (n=116, 95% CI)

Test	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Accuracy	P Value
NS 1 antigen ELISA	46.15% (30.07-62.87)	93.51% (85.50 -97.86)	78.26% (56.26-92.53)	77.41% (67.63-85.46)	77.58%	<0.0001
NS1 antigen ELISA and igM ELISA	71.79% (55.09-85.02)	84.42% (74.38-91.68)	70% (53.51-83.42)	85.53% (75.54-92.54)	80.17%	<0.0001

Results

A total of 181 patients were tested. Seventy two were less than 14 years of age and rest 109 were adults. One hundred and twenty-four (68%) patients were males. Seventy two patients were positive by any of the three tests i.e. PCR, NS1antigen ELISA or IgM ELISA. PCR was positive in 53 samples, NS1 antigen was detected 33 samples and IgM antibodies were found positive in 44 samples. Twenty-four out of 181cases were positive by both PCR and NS1antigen ELISA. Twenty-nine cases were only PCR positive and 24 were positive by NS1antigen ELISA. Of the 181 patients, 116 presented within first five days of fever. Of these, 18 were positive by both PCR and NS1antigen ELISA, 21 were detected positive by PCR only and 5 by NS1antigen ELISA only. Twenty-seven samples were positive by IgM ELISA. Table no.1 shows the accuracy indices of NS1antigen ELISA alone and in combination with IgM ELISA in comparison with PCR.

Discussion

Of the many epidemics reported from India, the one in 1996 reported almost 16,500 cases and it claimed 545 lives.[1] Since last decade dengue has assumed a pan-Indian proportion with all the serotypes of dengue in co-circulation and India belongs to category A of hyper endemic countries according WHO.[1] Clinical diagnosis of dengue infection is not reliable because of its protean manifestation. This makes early and accurate laboratory diagnosis essential for its timely management and prevention of mortality. RT –PCR which detects viral genomic sequence is highly sensitive (80-90%), but is expensive and not readily available in most of the hospital setups.[5] It is less sensitive at the time of defervescence than the acute viremic phase of illness. In present study, 53(out of 181) patients were detected positive by RT-PCR. The prevalence of dengue in our study by PCR was 29.28% (53/181).various studies across India have reported prevalence ranging from 10% to 58%.[6, 7, 8] NS1 antigen levels correlate well with viremia and it circulates at high levels during first few days of illness especially in patients with DHF.[9] NS1 antigen remains circulating in patients' blood for longer period than does viral RNA and is reported to be detectable even up to 14th day of illness.[10, 11] Thus NS1 antigen detection can be used for diagnosis of dengue up to two weeks of infection. Several NS1 antigen detection studies have reported sensitivity ranging from 22% to almost 93% and specificities of 98 to 100%.[9, 12 13] Serum samples of patients presenting up to first five days of illness in the present study demonstrated a sensitivity and specificity of NS1 antigen ELISA in comparison with PCR to be 46.15% and 93.51% respectively. The sensitivity of NS1 detection was significantly low (p value < 0.0001). NS1antigen detection methods are having more sensitivity in detecting patients with acute primary infections than secondary infection.[12] Differentiation between primary and secondary dengue was not possible in this study. Use of dissociated ELISA could have increased the NS1 detection in later stage of presentation where the antibodies can form complexes with antigen.[11, 13, 14] this can be possible explanation for low sensitivity of NS1 ELISA in our study. In patients with up to 5 days of fever, when the results of NS1 antigen ELISA in combination with IgM antibody ELISA were compared with PCR, sensitivity of combination was found to be 72%, which was significantly low(p value <0.0001). The sensitivity of NS1antigen ELISA alone or in combination with IgM ELISA was significantly low in comparison with PCR; still the test carries importance in setups lacking molecular diagnostic facilities for early confirmation of the diagnosis. Both the tests can be carried out in laboratories having ELISA facility. In patients presenting with more than five days of fever, PCR was positive in 14/65(21.53%), NS1antigen in 10/65(15.38%) and IgM antibody in 23/65(35.38%) cases. In developing countries most of the patients visit health care facility at later (more than 5 days) stage of

illness when antibody detection remains the choice of test. Demonstration of fourfold rise in titers in antibodies can confirm the diagnosis. But it becomes difficult to get a paired serum sample as patients rarely come for follow up visits after symptomatic relief. In a single serum sample the false positive results cannot be ruled out which is quite possible if patient has any similar viral infections like Japanese encephalitis. To rule out this one may need to do more specific test like NS1antigen ELISA in these cases. To conclude, PCR should be the preferred test to diagnose dengue infection in patients with less than 5 days of fever. Neither NS1antigen ELISA alone nor its combined use with IgM ELISA is good enough to replace PCR. In setups lacking PCR facility NS1 antigen ELISA can be used to confirm the diagnosis at early stage of the disease, though negative results cannot exclude the diagnosis.

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