Original Article

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Comparative Study of Antigen Detection and Viral Nucleic Acid Assay for Early Detection of Dengue Infection

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ABSTRACT

Background : *Differentiating dengue patients from other acute febrile illness patients is a great challenge among physicians. Thus early and rapid diagnosis of dengue virus infection is of top most importance. The present study was designed to establish an early and rapid diagnosis of dengue virus infection from single serum samples with comparison between two methods-antigen detection by Immunochromatographic test (ICT) and nucleic acid assay by Real time RT –PCR.*

Materials and methods : *This cross sectional study was carried out in the Department of Microbiology, Chittagong Medical College and Chevron Laboratory in Chattogram during the period of January 2012 to December 2012. A total 115 clinically suspected dengue patient of all ages and both sexex from indoor and Out Patient Department (OPD) of Chittagong Medical College Hospital were enrolled in this study.*

Results : *These test were done in 2-7 days of fever. Blood samples from 115 clinically suspected dengue cases had been taken. Among them, NS1 antigen by ICT were found positive in 78(67.8 %) cases and DENV RNA by real-time RT-PCR in 64 (55.7%) cases. Among 64 dengue PCR positive cases, dengue ICT was found positive in 61 (95.30%) cases & not detectable in 03 (4.65%) cases and in 51 PCR negative cases, 17 were found positive by ICT. The difference in diagnosing dengue by ICT and PCR was found highly significant, p < 0.001. Highest positivity of both test were observed in 2nd to 4th days. In this study, dengue ICT was detected 19 (70%) cases in • 5 days of fever where as PCR was detected only 02 (6.80%) cases. The sensivity , specificity , positive predictive value , negative predictive value of ICT in comparison to PCR were found 95.3% , 66.7%, 78.2 %, 91.9% respectively.*

Conclusion : *In the comparative evaluation, NS1 Antigen was found to be far more sensitive than Real time RT-PCR*

Key words : Antigen detection; Dengue fever; Immunochromatographic Test (ICT); Nucleic acid.

Introduction

Dengue is considered to be one of the most notable viral infections that may appear in the form of an endemic or epidemic febrile illness. It is transmitted by Aedes aegypti / Aedes albopictus mosquitoes which are present in most tropical and subtropical countries of the world.[1] There are four entities, which comprises the Dengue syndrome. These are Undifferentiated Fever (UF), Dengue Fever (DF), Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). Dengue Virus (DENV) are a group of 4 closely related but antigenically distinct serotype designated as DEN-1, DEN -2, DEN-3 and DEN-4.[2] Infection in human by one serotype produces life-long immunity against re-infection by the same serotype but only temporary and partial protection against the other serotypes.[3] A person can eventually be infected by all 4 serotypes. Several serotypes can be in the circulation during an epidemic.[4] Mortality rate < 1% when treated and DHF has a mortality rate 2-5 %. In untreated cases fatality rate may be as high as 30-40 %.[5] In 20-30% of DHF cases, patient develop DSS. Worldwide, children younger than 15 years constitute 90% of DHF patients.[6] The geographical distribution is around the equator with 70% of the total 2.5 billion people living in endemic areas from Asia and the Pacific.[7] In 2011, above 1 million dengue cases were reported to the Pan American Health Organization (PAHO) including 18,321 severe dengue cases and 716 deaths. In South pacific and SEA, 1,638 dengue cases have been reported. In W.Pacific region, 3, 53907 dengue cases were reported of which 1073 died.[8]

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In Bangladesh, dengue was first reported in 1964, but has maintained lower prevalence than most Southeast Asian states. Recently, however, Bangladesh has experienced an increase in dengue outbreaks, in 2017, the country reported 2,769 cases and in 2018, annual incidence increased to 10,148 cases. In 2019, the Directorate General of Health Services reported an incidence rate 10 times higher than the previous year, with 101,354 cases and at least 179 deaths. This outbreak had the highest case burden among all dengue outbreaks in Bangladesh. DENV-3 was described as the dominant serotype in the 2019 outbreak.[9]

Molecular diagnosis based on real time reverse transcriptase polymerase chain reaction, Real time RT – PCR is a nucleic acid detection assay has in acute phase serum sample with high sensivity (98.18%) and high specificity (100%). In negative ICT for NS1 antigen, there is a possibility that at least some of them might have had a dengue infection. Therefore, it is possible that it may have underestimated the number of patients who actually did have a dengue infection. In 2010, a study in Singapore, the sensivity of RT-PCR tend to decline after 3 days of the onset of fever, indicating that PCR technology would be effective for dengue diagnosis only during the very early stages of the disease .NS1 Antigen detection assay showed consistently high sensivity among sera collected within the first 6 days of fever.[8] Real time RT-PCR is only successful in the early phase of illness as dengue viremia is short -lived. In the comparative evaluation, there were several studies showed that NS1 antigen was found to be far more sensitive than real time RT-PCR as it depends upon duration of illness.[10]

The clinical diagnosis of dengue is not reliable due to the fact that this infection could result in asymptomatic or mild, undifferentiated fever.[11] As cross reactivity among flaviviruses in serological assays and anamnestic antibody response in secondary DEN virus infections, antibody detection assays are not adequate diagnostic tools and not specific like NS1 antigen and RNA detection methods. The dengue NS1 Antigen was not found in patient with Japanese encephalitis virus or yellow fever virus infection. Thereby there is no cross reaction of NS1 protein with those of other related flavivirus. Thus detection of NS1 has been a promising test to diagnose dengue in its early febrile stages. [12,10.]

However, PCR require specialized equipment, little progress in the standardization of protocols, which has limited their utility in lower socio-economic countries where there is a need for simple and affordable testing. So, this study has been designed to detect NS1 antigen by ICT and compare it with Dengue RNA by Real time PCR & tried to find out the effective method for early diagnosis of acute dengue infection . Moreover there is no such study in Chittagong.

Evaluation of NS1 antigen in comparison to nucleic acid detection in early diagnosis of Dengue fever.

Materials and methods

This cross sectional study was carried out in the Department of Microbiology, Chittagong Medical College (CMC) and Chevron Laboratory in Chattogram during the period of January 2012 to December 2012. A total 115 clinically suspected dengue patient of all ages and both sexes from indoor and Out Patient Department (OPD) of Chittagong medical college hospital were enrolled in this study.

Inclusion criteria:

- Clinically suspected patients were selected according to WHO criteria for case definition of dengue fever, dengue hemorrhagic fever, dengue shock syndrome.[14]
- Fever (2-7 days), temperature more than 38° C with two or more of the followings:- Severe headache/ Retro-orbital pain/Severe myalgia/ arthalgia/ back pain/Nausea/vomiting / abdominal pain/ Leucopenia/ Hemorrhagic manifestations evidenced through one or more of the followings :-

Positive tourniquet test /Petechiae. Ecchymosis. Purpura/ Bleeding from mucosa ie. Gum bleeding, epistaxis/Bleeding from injection or other site /Haematemesis, melena, hematuria, PV bleeding/ Thrombocytopenia/ Evidence of plasma leakage due to increase capillary permeability/.

- Evidence of circulatory failure manifested with one more of the following features : Hypotension for age and sex/ .Cold clammy skin and restlessness/ rapid and weak pulse/ Narrow pulse pressure (< 20 mm) /Profound shock

Exclusion criteria:

- Fever > 7 days/ Febrile case with definitive source of infection. e.g Respiratory tract infection/Urinary tract infection/Meningitis H/O bleeding tendency since birth/Patient receiving immunosuppressive therapy.

With all aseptic precautions, blood samples were collected (About 5 ml) from patients after taking informed written consent from patient or his / her legal guardian and processed according to standard laboratory procedure.

Rapid Test (One step Dengue NS1 Ag Test) [Standard diagnostics, Korea] was done and serum stored -70^{0}c. Then Real time PCR assay for Dengue viral RNA (Advanced Kit 150 test) (genesig, PrimerDesign™ Ltd, U.k) had been done.

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Results

In the present study, among the 115 cases, NS1 antigen by ICT showed positive in 78 (67.8 %) cases (Figure 1).

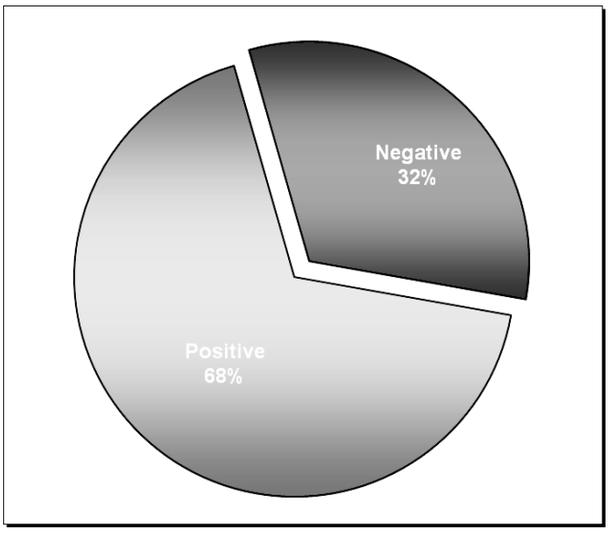

Figure 1 Detection of NS1 Ag by ICT in dengue infection (n = 115)

Figure 2 showed that 64(55.7%) cases were positive by real-time RT-PCR .

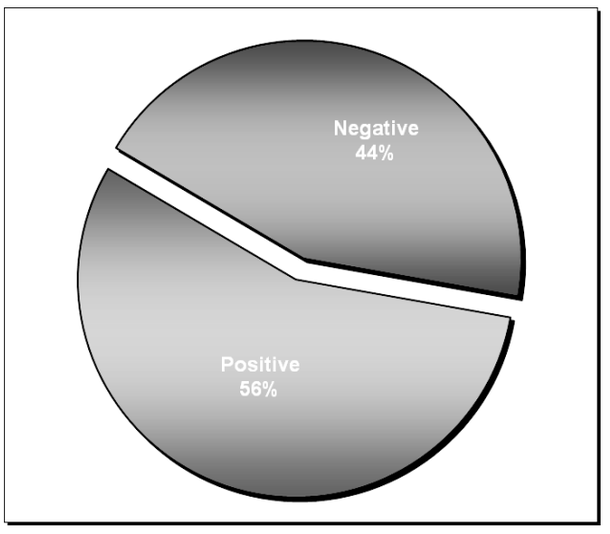

Figure 2 Detection of viral RNA by Real time RT-PCR (n = 115)

Comparison of ICT with PCR showed that among 64 PCR positive cases, 61 (95.3%) were ICT positive, among 51 PCR negative cases, 17(33.3%) cases were positive by ICT. Differences found staistically highly significant $p < 0.001$) [Table I]

Table I Comparison between Dengue PCR and Dengue ICT

Dengue PCR	Dengue ICT		Total
	Positive	Negative	
Positive	61 (95.3)	03 (4.6)	64
Negative	17 (33.3)	34 (66.6)	51
Total	78	37	115

- Figures within parentheses indicate percentages.
- $\chi^2 = 47.163$, p = 0.000, Highly Significant ($P < 0.001$)

Table II showed Dengue ICT and PCR in different durations of fever, ICT showed highest positivity on 3rd day 72.2 %, on 6th day 70% and least on day 5th 55.6% and PCR showed highest positivity on 2nd day 77.3 % , on 3rd day 72.2 % and least 22.2% on 5th day.

Table II Distribution of positive case detection according to duration of fever

Duration of Fever	Dengue ICT Positive		Dengue PCR Positive	
	n	%	n	%
2 Days (n = 22)	15	68.2	17	77.3
3 Days (n = 36)	26	72.2	26	72.2
4 Days (n = 28)	18	64.3	19	67.9
5 Days (n = 09)	05	55.6	02	22.2
6 Days (n = 20)	14	70.0	00	00.0
Total (n = 115)	78	67.8	64	55.7

In <5 days of fever Dengue ICT were positive 59 (68.60 %) cases and in ≥ 5 days 19 (70%) whereas Dengue PCR were found positive 62 (72.09%) cases in < 5 days of fever and 02 (6.80 %) in 5 days [Table III]

Table III Relation of fever between ICT and PCR in < 5 days and ≥ 5days

Duration of fever	ICT Positive	PCR Positive
< 5 days (n=86)	59 (68.60 %)	62(72.09 %)
≥ 5 days (n=29)	19 (65.51%)	02(06.80%)

Table IV showed that sensivity, specificity, positive predictive value, negative predictive value of ICT in comparison to PCR were 95.3%, 66.7%, 78.2%, 91.9% respectively.

Table IV Evaluation of ICT in respect to PCR as diagnostic test

Validity of ICT	
Sensitivity	95.3%
Specificity	66.7%
Positive Predictive Value	78.2%
Negative Predictive Value	91.9%

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Discussion

Dengue Fever and Dengue Haemorrhagic Fever (DF/DHF) has emerged as the most important arboviral disease of mankind in terms of both morbidity and mortality.[14] Although dengue fever is a self limiting disease, DHF may lead to disordered homeostasis including vascular changes, thrombocytopenia, coagulation disorder and occasionally circulatory failure i.e Dengue Shock Syndrome (DSS) with 30-40 % fatality rates in untreated cases.[16] Transmission of dengue is now present in every region of the world and more than 125 countries are known to be dengue endemic.[16]

In the present study, 115 clinically suspected dengue cases of all age groups and sex within 2-7 days of fever were studied. Out of which 78(67.8 %) cases were positive by NS1 Ag and 64(55.7%) cases were by RT-PCR analysis. Similarly to present study conducted by Wang et al in 2009 who found that 64% tested positive for NS1 antigen and 50.31% by RT-PCR in clinically suspected cases.[17]

Among 64 dengue PCR positive cases, dengue ICT was found positive in 61 (95.30%) cases and not detectable in 03 (4.65%) cases and in 51 PCR negative cases 17 cases were found positive by ICT. Difference in diagnosing dengue between ICT and PCR was found highly significant ($p < 0.001$). Nearly similar to present study, Lai et al in 2007 found 18 sera collected between 4th to 7th days of fever which were RT-PCR negative but were positive by NS1 assays in comparison. He also found eight sera collected within first 3 days of fever that were positive for dengue by RT-PCR but were negative by NS1 assays.[18]

In the present study, RT-PCR was found highest 77.3% cases on 2nd day, 72.2% cases on 3rd day , 67.9% cases on 4th day ,22.2% cases on 5th day and no cases on 6th day. Closely similar to present study in 2010, Huhtamo et al in Finland, found highest RT-PCR positivity 86.7 % on days 1-3 followed by 76.9% on days 4-5 and 63.2 % on days 6-7 after onset of fever.[19] In the study highest percentage of NS1 antigen positivity 26 (72.2 %) were found on 3rd day followed by 14 (70%) on 6th day, 15 (68.2%) on 2nd day, 18 (64.3%) on 4th day and least 05 (55.6%) on 5th day. Our result closely related with Huhtamo et al. in 2010 found highest percentages of NS1 antigen positive samples 84.2 % were found on days 6-7 after onset of symptoms followed by 78.6% on days 1-3, 74.1% on day 4-5 and 70.6 % on day 8 or later.[19] A possible explanation for reduced NS1 sensivity in the presence of a measurable anti-DENV antibody, inappropriate temperature storage of samples during transportation because more than 95% of samples were sent at ambient temperature.[17] Furthermore the negative result of pcr day be due to some of patients may be in the late viremic or post-viremic phase.,due to failure to maintain the cold chain during transport of the samples, resulting in virus inactivation.[20]

In the present study, Dengue ICT were found positive in 59 (68.60 %) cases in < 5 days of fever whereas 19 (70%) cases were present in ≥5 days .Similar to present study the dengue NS1 antigen assays evaluated in Singapore in 2010, 71.2 % - 82.7 % sensivity of detecting dengue in acute sera collected within the first 3 days of fever. There sensivity remain persistently high (66.7-79.2%) until the sixth day after the onset of fever.[16] Dengue PCR were found positive in 62(72.09%) in < 5 days of fever whereas only 02 (6.80 %) in ≥5 days. Higher detection rate by the 2 test was found during the 2nd to 4th days. Shekaron et al in 2010 found that NS1 could be detected upto day 14 from the onset of fever while RT-PCR was only able to detect viral RNA up to 8 day post infection.[21] NS1 Ag is present in the serum during viremia.[22] Study conducted in Finland in 2009 found NS1 antigen remain detectable later than the viral RNA and negative RNA results might be due to RNA degradation in samples.[19] A study conducted in Malaysia by Kassim et al in 2011 who found that DENV antigens from as early as Day 2 up to Day 9 of fever.[23] Antigen detection was highest between Days 3 and 4 with a detection rate ranging from 10.4% to 12.8%. Both antigen and PCR tests showed a decreased detection rate to 8.8% and 7.2%, respectively on Day 5 of fever.[23]

In the present study sensivity, specificity, positive predictive value, negative predictive value of ICT in comparison to PCR were 95.3%, 66.7%, 78.2%, 91.9% respectively. Study in Thailand in 2011, the NS1 rapid test was compared with semi-nested PCR, with sensitivity of 70.6% and specificity of 73.4%. Furthermore positive and negative predictive values for the NS1 rapid test was calculated and shown to be 69.4% and 74.6%, respectively.[24] However, sensitivities varying from 63.2% to 93.3% have also been reported for this kit.[25] Although dengue NS1 antigen detections up to the 9th day are described, present study was analyzed cases only up to the 6th day due to the low number of samples representing after 6th days in our population. The lack of later samples in this study did not allow us to determine when NS1 detection would decrease. However, various studies found NS1 antigen in 82% to 83% of patients with dengue from day 1 to 9th after the onset of fever.[26]

On days 6-7 NS1 antigen performed superior to RNA detection [20]. In this study, comparison of antigen detection by ICT and viral nucleic acid detection by Real time RT-PCR will provide an actual conception

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about the role of antigen based ICT test for early diagnosis of dengue infection over RT-PCR which will help to minimize the costs as well as dengue related mortality.Currently, there is no single diagnostic assay that can accurately detect dengue throughout the acute and convalescence phases.Though highly specific and sensitive especially during the early phase of the clinical disease, RT-PCR requires skilled personnel, sophisticated equipments and laboratory facilities. As the majority of primary healthcare settings lack the opportunity to perform RT-PCR, the routine diagnosis of dengue fever mainly relies upon the rapid diagnostics test assay.[27]

Limitations

The study was conducted at a very short period of time and moreover small sample size was also a limitation of the study.

Conclusion

Dengue infection presents with non specific fever that mimics other viral illness. ICT can detect dengue infection after 5 days of illness by detecting NS1 Ag where PCR may be negative. Moreover, the test has similar sensivity with that of PCR and is relatively simple, rapid, cost effective, less time consuming and can be done in field level. This does not require skilled personnel to perform the test. Bangladesh, many patients refuse to do PCR as they cannot afford to bear the cost of these tests. Based on this observation, dengue NS1 Antigen assays appeared to be alternative to RT-PCR and antibody testing in the routine diagnosis of dengue

Recommendation

Nucleic acid based assay are promising tools to permit virus serotyping and to monitor the evolution of geographic strain of dengue virus.

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Disclosure

All the authors declared no competing interest.

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