Original article

Comparison between serological & molecular methods for diagnosis of dengue fever and its correlation with duration of illness

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Abstract

Introduction: Dengue is caused by Dengue virus (DENV), a mosquito-borne flavivirus.DENV causes a wide range of diseases in humans, from a self-limited Dengue Fever (DF) to a life-threatening syndrome called Dengue Haemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS).

Materials and methods: 426 clinically suspected cases of Dengue fever were included in this study. As per the duration of fever; Samples were separated in four groups Group A: fever up to 3 days, Group B: fever from 4 days to 6 days ,Group C: fever from 7 days to 9 days and Group D: fever from 10 days and more.days and Group D: fever from 10 days and more .Samples from Group A, B, C were subjected to serological tests and molecular tests while samples from Group D are subjected to serological tests only; as theoretically molecular tests are negative after 7 days of fever

Results : Out of 426 samples 97 (22.8%) were positive by NS1 Ag, 253 (59.4%) by Rapid Test, 270 (63.4%) by ELISA (IgM & IgG) and 78 (18.3%) by RT-PCR.Sensitivity of NS1 with respect to PCR: 73.5% , Specificity of NS1 with respect to PCR: 91.5%.Sensitivity of Rapid Anti dengue IgM test: 86.4 % .Specificity of Rapid Anti dengue IgM test: 90.8 %.

Discussion : 327/426 (76.8%) clinically suspected Dengue cases confirmed by Serological and Molecular methods. 78 (18.3%) sample were positive by RT-PCR for Dengue RNA .97 (22.8%) samples were positive for NS1 antigen test. .270 (63.4%) sample were positive by ELISA for IgM & IgG antibodies. 253 (59.4%) sample were positive by Rapid test for IgM & IgG antibodies.With use of appropriate test; diagnosis of dengue fever can be made early and prompt treatment can be provided to the patient as a result cost of illness due to testing, treatment and duration of hospitalisation can be reduced and valuable time can be saved and number of patient developing complication (DHF and DSS) can be reduced.

Key words: Dengue, Molecular method

Introduction:

Dengue fever is the most rapidly spreading mosquito-borne viral disease in the world. An estimated 50 million infections per year occur across approximately 100 countries. Incidence has increased 30-fold with increasing geographic expansion with potential for further spread. [1,2,3]

India is endemic for DF and DHF. All the four serotypes are found in the country. Case fatality rates in endemic countries are 2.5%. [4] During epidemics of dengue, attack rates among susceptible are 40-90%. [5, 6]

Early laboratory diagnosis of acute dengue virus infection still remains a problem. [11] At present, the three basic methods used by most laboratories for the diagnosis of dengue virus infection are viral isolation and identification, detection of viral genomic sequence by a nucleic acid amplification technology assay (RT-PCR), and detection of dengue virus-specific IgM antibodies by the IgM-capture enzyme-linked immune-sorbent assay (MAC-ELISA) and/or the rapid dengue Immunochromatographic test (DIT). [11] Though virus isolation and characterisation are considered as the gold standard of laboratory diagnosis for acute dengue virus infection, it is expensive and it takes at least 6–10 days for the virus to replicate in tissue cell culture or laboratory mosquitoes. [12]

In the view of this, present study intended to determine the effectiveness of various serological tests (NS1 antigen test, Rapid Igm / IgG test, MAC ELISA and IgG ELISA) and molecular test available for diagnosis of DNV infection and correlating it with clinical course of illness.

Materials and methods

This prospective study was carried out over a period of year in a Tertiary care hospital.426 clinically suspected cases of Dengue fever were included in this study. Institutional Ethics Committee approval was taken. An informed consent of patients was obtained during sample collection. Patients included were showing following symptoms & sign were: High fever and at least two of the following: Severe headache ,Severe eye pain (behind eyes) ,Joint pain, Muscle and/or bone pain, Rash, Mild bleeding manifestation, Low white cell count. [2]

One set of sample was collected from each patient which include: 5ml Serum sample for serological tests and 3-5ml whole blood in EDTA for Molecular study.

As per the duration of fever; Samples were separated in four groups Group A: fever up to 3 days, Group B: fever from 4 days to 6 days ,Group C: fever from 7 days to 9 days and Group D: fever from 10 days and more

Samples from Group A, B, C were subjected to serological tests and molecular tests while samples from Group D are subjected to serological tests only; as theoretically molecular tests are negative after 7 days of fever & for economic reasons.

Serological Test performed on the samples were **Rapid NS1 Antigen test (SD Bioline** colloid gold based immunochromatography test to detect dengue virus NS1 Ag in human serum or plasma),**Rapid IgM / IgG test (SD Bioline** solid phase immunochromatographic) **MAC ELISA:** SD Bioline (MAC ELISA), Korea , **IgG ELISA:**SD Bioline (GAC ELISA), Korea .

Molecular Test : RNA extraction & Real time Polymerase Chain Reaction (RT PCR) (TaqMan –Roche system)

Results:

A total of 426 patients with clinical features suggestive of dengue infections were included in the study. Dengue infections were confirmed in 327 (76.8%) of the patients either by serology or PCR. Out of 426 samples 97 (22.8%) were positive by NS1 Ag, 253 (59.4%) by Rapid Test, 270 (63.4%) by ELISA (IgM & IgG) and 78 (18.3%) by RT-PCR. (Table I)

Sensitivity of NS1 with respect to PCR: 73.5% , Specificity of NS1 with respect to PCR:

91.5% Positive Predictive Value of NS1 with respect to PCR: 78.2% ,P= 5.51 (significant) . PCR was taken as gold standard. (Table II)

Sensitivity of Rapid Anti dengue IgM test: 86.4 % .Specificity of Rapid Anti dengue IgM test: 90.8 % , Positive Predictive Value of Rapid Anti dengue IgM test: 86.9 % (Table III)

Sensitivity of Rapid Anti dengue IgG test: 90.3 % ,Specificity of Rapid Anti dengue IgG test: 89.6 %. Positive Predictive Value of Rapid Anti dengue IgG test: 87 % Table IV

Detection rate of DNV infection in the early phase (Group A and B) increased by combining NS1 detection with antibodies detection tests. Table V

Group A (Fever 1 -3 day): out of the 92 sample tested were positive by NS1 Ag test in 31 (33.7%), IgM Rapid in 12 (13%), IgG Rapid in 6 (6.5%), Rapid IgM & IgG both in 1 (1.1%), IgM ELISA in 10 (10.9%), IgG ELISA in 7 (7.6%) and Both (IgM & IgG) in 5 (5.4%) samples. (Table VI)

Group B (Fever 4 -6 day): out of the 167 sample tested were positive by NS1 Ag test in 45 (26.9%), IgM Rapid in 43 (25.7%), IgG Rapid in 16 (9.6%), Rapid IgM & IgG both in 42 (25.1%), IgM ELISA in 53 (31.7%), IgG ELISA in 20 (12%) and Both (IgM & IgG) in 38 (22.8%) samples. (Table VI)

Group C (Fever 7 –9 day): out of the 109 sample tested were positive by NS1 Ag test in 18 (16.5 %), IgM Rapid in 9 (8.3%), IgG Rapid in 32 (29.4%), Rapid IgM & IgG both in 49 (44.9%), IgM ELISA in 11 (10.1%), IgG ELISA in 40 (36.7%) and Both (IgM & IgG) in 41 (37.6%) samples. (Table VI)

Group D (Fever ≥ 10 day): out of the 58 sample tested were positive by NS1 Ag test in 3 (5.2%), IgM Rapid in 1 (1.7%), IgG Rapid in 22 (37.9%), Rapid IgM & IgG both in 14 (24.1%), IgM ELISA in 2 (3.4%), IgG ELISA in 27 (46.6%) and Both (IgM & IgG) in 14 (24.1%) samples. (Table VI)

In the present study virus is identified in 78 (18.3%) of the patients by RT-PCR whereas NS1 antigen detected in 97 (22.8%) cases. RT-PCR positivity is higher in early phase of illness i.e. up to 5 day of illness which is similar to NS1 Antigen detection test which has higher positivity in early phase of illness but may be detected up to 10 days of illness. **Table VII**

Table I

Total Sample tested	**Dengue Positive (%)**			
	NS1	**Rapid Test**	**ELISA**	**RT-PCR**
426	97 (22.8)	253 (59.4)	270 (63.4)	78 (18.3)

Table II (n = 283)

		Dengue PCR	
		Positive (*n*=78)	Negative (*n*=205)
Dengue NS1 antigen	Positive (n=83)	61	22
	Negative (*n*=200)	17	183

Table III

		Dengue IgM ELISA	
		Positive (*n*=176)	Negative (*n*=250)
Rapid Anti Dengue IgM	Positive (n=177)	153	24
	Negative (*n*=249)	23	226

Table IV

		Dengue IgG ELISA	
		Positive (*n*=193)	Negative (*n*=233)
Rapid Anti Dengue IgG	Positive (n=186)	168	18
	Negative (*n*=240)	25	215

Table V

Group	NS1 Only	NS1 + IgM/IgG or Both
A	33.7 %	45.7 %
B	26.9 %	74.3 %
C	16.5 %	89 %
D	5.2 %	67 %

Table VI

Days of Fever	Total	NS1 detected Number (%)	IgM only Number (%)		IgG only Number (%)		Both IgM and IgG Positive Number (%)	
			Rapid	ELISA	Rapid	ELISA	Rapid	ELISA
1-3	92	31 (33.7)	12 (13)	10 (10.9)	6(6.5)	7(7.6)	1(1.1)	5 (5.4)
4-6	167	45 (26.9)	43 (25.7)	53 (31.7)	16(9.6)	20(12)	42(25.1)	38 (22.8)
7-9	109	18 (16.5)	9 (8.3)	11 (10.1)	32(29.4)	40(36.7)	49(44.9)	41 (37.6)
≥ 10	58	3 (5.2)	1 (1.7)	2 (3.4)	22(37.9)	27(46.6)	14(24.1)	14 (24.1)

Table VII

	Total	**NS1 (%)**	**PCR (%)**
Fever 1-3	92	31 (33.7)	28 (30.4)
Fever 4-6	167	45 (26.9)	45 (26.9)
Fever 7-9	109	18 (16.5)	5 (4.6)
Fever ≥ 10	58	3 (5.2)	ND
Total	**426**	**97 (22.8)**	**78 (18.3)**

Table VIII

Duration of fever/ days	**Total number tested**	**Rapid test No. positive (%)**				**ELISA No. positive (%)**			**RT-PCR No. positive (%)**
		NS1 (%)	**Total positive (IgM/ IgG or Both)**	**IgM**	**IgG**	**Total positive (IgM/IgG or Both)**	**IgM**	**IgG**	
1	28	0 (0)	0(0)	0	0	0(0)	0	0	0(0)
2	34	19 (55.9)	7 (20.9)	7	3	8 (23.5)	5	6	17(50)
3	30	12 (40)	12 (40)	9	4	14 (46.7)	10	6	11(36.7)
4	73	18 (24.7)	48 (65.8)	40	22	50 (68.5)	45	22	20(27.4)
5	65	21 (32.3)	39 (60)	30	24	40 (61.5)	30	23	18(27.7)
6	29	6 (20.7)	20 (69)	18	16	21 (72.4)	18	14	7(

7	24	7 (29.2)	16 (66.7)	8	13	15 (70.8)	7	12	5(20.8)
8	60	11 (18.3)	52 (86.7)	39	46	53 (88.3)	35	46	ND
9	25	0 (0)	22 (88)	11	22	24 (96)	10	23	ND
≥10	58	3 (5.2)	37 (63.8)	15	36	43 (74.1)	16	41	ND
Total	**426**	**97 (22.8)**	**253 (59.4)**	**177**	**186**	**270 (63.4)**	**176 (41.3)**	**193 (45.3)**	**78 (18.3)**

Discussion

Dengue virus infection has emerged as the most important and widely spread arboviral disease in the world.

In order to provide timely information for the management of the patients, and early public health control of dengue outbreaks, it is important to establish a diagnosis of acute dengue virus infection during the first few days after manifestation of clinical symptoms. Detection of viral genomic sequence by RT-PCR is also an expensive method and is not widely available in most hospital diagnostic laboratories. The third method, assay of anti-dengue specific IgM and IgG depends on the time taken for an infected person's immunological response to produce IgM and IgG antibodies against dengue virus antigens.

Due to the higher mortality associated with secondary infections, it is important to use diagnostic assays that are able to differentiate between the two forms of dengue virus infection In endemic areas, secondary infections are most common, as the majority of children have antibodies against dengue by the time they are 5 years of age. As primary and secondary dengue virus infections show markedly different immunological responses, the detection of antibodies is a valuable procedure to diagnose and differentiate dengue virus infections.

NS1 Antigen detection was compared with RT-PCR for Dengue RNA by Taqman Roche System. NS1 Antigen detection test shown sensitivity of 73.5% and specificity of 91.5% (table 6).(7)

In group A the detection of Dengue fever by NS1 antigen test alone is 31 (33.7%) and with either IgM or IgG antibodies or both is 30 (32.6%) which shows that NS1 detection have high diagnostic value in early phase i.e. 1-3 days of illness and combination of NS1 with either of the antibodies detection tests enhance the detection rate from 33.7% to 45.7%. (Table 8)

In group 2 where anti-dengue antibodies s1tart appearing NS1 antigen detection rate decreases from 33.7% in Group A to 26.9% in group B. Whereas combination of NS1 antigen tests with antibody testing increases dengue diagnosis from 26.9% by NS1 anti¡ alone to 74.3%. (Table 8)

In Group C and D detection rate antigen alone reduces drastically to 16.5% and 5.2% respectively. ( ) This was corresponding to other study by Kao *et al*,2005

Data obtained in this study show NS1 antigen were detected from as early as Day 2 (55.2% samples) up to Day 10 (5.2% samples) of fever. These findings are comparable to a study by Alcon *et al* (2006) who recovered NS1 antigen until Day 9 of symptoms with NS1 detection rate of 80% upto Day 6.

Low detection rates with NS1 antigen observed in present study may be linked to immune complexes found in samples collected.

Anti-Dengue antibodies were detectable as early as second day of fever and onwards may be due to subclinical infection.

Rapid test have shown sensitivity of 86.4% and specificity 90.8% with respect to ELISA for IgM. Similarly sensitivity and specificity for IgG found to be 90.3% & 89.6% respectively. (7).

It was found that in the early phase of illness i.e. Day 1-6 NS1 antigen test and RT-PCR results were similar to each other. As the duration of fever increases NS1 antigen still detectable but RT-PCR shown decreased results from Day 6 onwards. (Table 2)

IgM antibodies were detectable from Day 2 (20.1%) and rate of detection increases upto Day 6 (62%). The rate then starts decreasing and becomes 20% by Day 10. (Table 2)

IgG antibodies were detected first on day 2 itself as DNV infection is endemic in India and most people were exposed to it and have immunological memory for it. Detection rate of IgG antibodies was higher from Day 5 (48%) and it rises by Day 10 (70.1%) (Table 2)

For confirming the diagnosis of DNV infection in early phase (Day 1-3) NS1 antigen test and RT-PCR both were equally effective (33.7% & 30.4% respectively). DNV infection confirmation rate can be increase by using combination of NS1 antigen test or RT -PCR with antibodies detection tests.

Day 4-6 of illness show higher diagnostic rate by antibodies test (66.5%) than NS1 antigen test (26.9%) and RT-PCR (26.9%) hence antibodies test were found to be better diagnostic tool for DNV infection in this phase of illness. (Table 2)

In Day 7-9 of illness RT-PCR not able to show presences of DNV in not more than 20% samples. NS1 antigen was still detectable; but in less than 9.8% samples.

IgM antibodies were detectable in 47.7% and IgG in 74.3% of samples. (Table 2)

Day 10 onwards NS 1 may be detected but it count for less than 5.2% of sample size. Whereas IgG was present in 70.7% samples and IgM in 27.6%

Conclusion

NS1 antigen detection has potential value for screening patient samples during the early acute phase (Day 1-3).NS1 antigen found to be equally effective for diagnosis of Dengue fever in early phase of illness (upto 3 days) can be a cheap replacement for expensive RT-PCR testing.

In the phase of day4 to 6 of illness NS1 detection test and antibodies test are equally effective can be used alone or in combination for higher specificity. In late stage of illness IgG detection can be a good diagnostic test (46.6%) Rapid antibody detecting tests are equally effective as ELISA test so can instead of cumbersome ELISA test.

With use of appropriate test; diagnosis of dengue fever can be made early and prompt treatment can be provided to the patient as a result cost of illness due to testing, treatment

and duration of hospitalisation can be reduced and valuable time can be saved and number of patient developing complication (DHF and DSS) can be reduced.

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