Journal of Cardiovascular Disease Research
ISSN: 0975-3583, 0976-2833 VOL09, ISSUE 4, 2018

EVALUATION OF THE EARLY DIAGNOSIS OF DENGUE WITH SEROLOGICAL MARKERS AND CLINICAL FEATURES IN AN INDIAN HEALTHCARE CENTER

Dr Abhishek S Goenka,[1] Dr. Chandan P. Borale[2*]

[1]Assistant Professor, Department of Microbiology, Government Medical College, Akola, Maharashtra

[2*]Assistant Professor, Department of Internal Medicine, Government Medical College, Akola, Maharashtra

Address for correspondence

Email id: boralechandan@gmail.com

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ABSTRACT

Background: In subtropical and tropical areas, arbovirus poses a major health risk and causes epidemics having significant negative social and economic effects along with high mortality and morbidity rates by DENV (dengue virus) transmitted to humans by Aedes family mosquitoes.

Aim: The present study aimed to assess the role of NS1 antigen in early detection of dengue virus versus IgM ELISA and to study clinical features in early-stage dengue infection.

Methods: The study assessed 50 adult subjects with clinical features suggestive of dengue infection. Subjects were interviewed for demographics including age, gender, and occupation. Subjects were then subjected to physical and systemic assessment along with clinical symptoms and signs. The findings were recorded for results formulation.

Results: 35 subjects tested positive for IgM ELISA, among these 32 tested positive for the NS1 antigen test and 3 subjects were negative. The sensitivity, specificity, positive, and negative predictive values were 92.84% and 90%. 95.57%, and 84.36% respectively with $p<0.01$.

Conclusions: The present study concludes that dengue infection that poses a major healthcare issue can be diagnosed early with clinical features such as SGOT>SGPT, thrombocytopenia, bleeding manifestations, myalgia, and retro-orbital pain which is supported by detecting NS1 antigen.

Keywords: Dengue virus, rapid diagnostic test, ELISA, thrombocytopenia, NS1 antigen

INTRODUCTION

In subtropical and tropical areas, arbovirus poses a major health risk and causes epidemics having significant negative social and economic effects along with high mortality and morbidity rates by DENV (dengue virus) transmitted to humans by Aedes family mosquitoes. The main virus transmitted by arthropods in humans is dengue fever affecting 50 million infectious cases annually and 500,000 hospitalizations with dengue hemorrhagic fever mostly in young subjects and case-fatality rates of >5% in few areas. Nearly 120 nations are affected by dengue fever epidemics and several with high incidence.[1]

Family Flaviviridae and genus Flavivirus contain DENV (dengue virus). Depending on the size of its positive sense single-stranded RNA of nearly 11kb, the virus is divided into 4 serotypes as

DENV-1, 2, 3, and 4 antigenic properties. DENV genome has 10 open-reading frames each of which is translated to polyprotein which broke by signal peptidase from the host cell into C, E, and M structural proteins and 7 non-structural proteins namely NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5. Clinical symptoms of infection by any of these serotypes are benign and generic till severe phases that occasionally present deadly repercussions such as DSS (dengue shock symptoms) and DHF (dengue hemorrhagic fever).[2]

Clinicians are not able to assess which subjects will have severe disease in the early febrile stage having symptoms such as rash, body aches, headache, malaise, and fever. During effervescence, symptoms suggestive of shock and plasma extravasation can be seen including fainting, temperature decrease associated with profuse perspiration, persistent vomiting, somnolence, restlessness, continuous and severe abdominal pain, clinical warning signs, 20% thrombocytopenia, and/or bleeding.[3]

IgG/IgM antibody detection is standard for serological confirmation of dengue fever. The presence of high IgG/IgM in acute serum from suspected dengue cases suggests probable dengue infection. Dengue confirmation is done by virus isolation, IgG/IgM seroconversion, and genome detection. Recently, NS1 antigen detection has been used for early dengue infection diagnosis. NS1 glycoprotein is produced by flavivirus and is vital for virus viability and replication. As NS1 is secreted in blood, virus tests were developed for diagnosis of DENV infection with NS1. These tests include assessment of NS1-specific IgG/IgM responses, lateral flow antigen detection, and antigen-capture ELISA.[4]

Presently, dengue NS1 antigen detection is used for early diagnosis of DENV infections. Hence, the present study aimed to assess the role of NS1 antigen in the early detection of dengue virus infection versus IgM ELISA and to study the clinical features in the early stage of dengue infection.

MATERIALS AND METHODS

The present prospective hospital-based observational study was aimed to assess the role of NS1 antigen in the early detection of dengue virus infection versus IgM ELISA and to study the clinical features in the early stage of dengue infection. The study subjects were from the Outpatient Department of the Government Medical College, Akola, Maharashtra. Verbal and written informed consent was taken from all the study subjects before participation.

The study included 50 adult subjects from both genders presenting with clinical features suggestive of dengue infection. The inclusion criteria for the study were subjects aged >12 years, fever history of >38ºC for < 7 days with 2/more signs and symptoms of bleeding manifestation, hypotension, rash, arthralgia, myalgia, retro-orbital pain, and/or headache. The exclusion criteria were subjects with localized infection sources.

All included subjects were interviewed to record demographics including age, gender, and occupation. A history of similar complaints in past and current treatment was noted. Subjects then underwent comprehensive physical examination, vitals such as respiratory rates, blood

pressure, and pulse rate with other clinical symptoms and signs of dengue fever. This was followed by a systemic assessment. All data were recorded on preformed structured proforma. Investigations included electrocardiography, Dengue IgM ELISA after 7 days, NS1 antigen, liver function test, renal function test, and complete blood counts from intravenous blood. The sensitivity and specificity were confirmed with IgM ELISA.

The data gathered were analyzed statistically using SPSS (Statistical Package for the Social Sciences) software version 16.0 (SPSS Inc., Chicago, USA) for assessment of descriptive measures and the Chi-square test. The results were expressed as mean and standard deviation and frequency and percentages. The p-value of <0.05 was considered statistically significant.

RESULTS

The present prospective hospital-based observational study was aimed to assess the role of NS1 antigen in the early detection of dengue virus infection versus IgM ELISA and to study the clinical features in the early stage of dengue infection. 35 subjects tested positive for IgM ELISA Among these 32 tested positive for the NS1 antigen test and 3 subjects were negative. There were 62% (n=31) males and 38% (n=19) females in the study. The majority of the subjects were in the age range of <30 years with 60% (n=30) subjects followed by 16% (n=8) subjects from 31-40 years, 10% (n=5) from 41-50, 12% (n=6) from 51-60, and 2% (n=1) from >60 years respectively (Table 1).

For clinical features of dengue fever assessed with NS1 antigen in subjects assessed till 7 days of fever onset, diarrhea was seen in 1 subject from NS1 positive and no subject from NS1 negative which was statistically non-significant with p=0.34. Vomiting also showed a non-significant association with NS1 positive status with p=0.07. Bleeding was significantly associated with NS1 positive status with p<0.001. Arthralgia and rash were significantly associated with NS1 positive status with p=0.003. Abdominal pain and headache showed non-significant association with NS1 status with p=0.07 and 0.14 respectively. Retro-orbital pain and myalgia showed significant association with NS1 positive status with p=0.01 (Table 2).

For blood counts, in TLC, leucocytosis, leucopenia, and normal counts were seen in 6% (n=2), 16% (n=8), and 78% (n=39) subjects respectively with mean TLC as 5500±2489.64. Normal platelet counts and thrombocytopenia were seen in 14% (n=7) and 86% (n=43) subjects respectively with a platelet count of 37120.55±32304.97. PCV% of >50 and <50 was seen in 12% (n=6) and 88% (n=44) subjects with a mean PCV of 42.42±8.32% (Table 3). NS1 negative and positive status was seen in 32% (n=16) and 68% (n=34) subjects respectively. IgM negative status was seen in 32 NS1 positive and 2 NS1 negative subjects. IgM positive status was seen in 35 NS1 positive and 15 NS1 negative subjects respectively. The sensitivity, specificity, positive predictive value, and negative predictive values were 92.84%, and 90%. 95.57%, and 84.36% respectively with p<0.01 (Table 4).

Concerning the correlation of the NS1 test to clinical findings, the statistically non-significant association was seen in CNS, hypotension, and pulse rate with p=0.13, 0.14, and 0.15

respectively. A significant association of the NS1 test to abdominal pain, edema, and icterus with p=0.003, 0.03, and 0.01 respectively (Table 5). For the correlation of blood investigations to the NS1 antigen test in study subjects, TLC showed a statistically non-significant association with NS1 antigen with p=0.12. A similar non-significant association was seen in NS1 antigen and PCV (%) with p=0.08. However, a significant association was seen in platelet counts and NS1 antigen test with p=0.002 (Table 6).

The study results showed that for the assessment and correlation of ECG and NS1 findings in study subjects. Sinus bradycardia was seen in 35.29% (n=12) NS1 positive subjects and 18.75% (n=3) NS1 negative subjects respectively. Normal ECG findings were seen in 64.70% (n=22) subjects with NS1 positive status and 81.25% (n=13) subjects with NS1 negative subjects. The difference was statistically non-significant with p=0.15 as shown in Table 7.

DISCUSSION

The present study assessed 50 subjects where 35 subjects tested positive for IgM ELISA Among these 32 tested positive for the NS1 antigen test and 3 subjects were negative. There were 62% (n=31) males and 38% (n=19) females in the study. The majority of the subjects were in the age range of <30 years with 60% (n=30) subjects followed by 16% (n=8) subjects from 31-40 years, 10% (n=5) from 41-50, 12% (n=6) from 51-60, and 2% (n=1) from >60 years respectively. These data were similar to the studies of Martinez TE[5] in 2006 and Guzman MG et al[6] in 2010 where authors assessed subjects with demographic data comparable to the present study.

On assessing the clinical features of dengue fever with NS1 antigen in subjects assessed till 7 days of fever onset, diarrhea was seen in 1 subject from NS1 positive and no subject from NS1 negative which was statistically non-significant with p=0.34. Vomiting also showed a non-significant association with NS1 positive status with p=0.07. Bleeding was significantly associated with NS1 positive status with p<0.001. Arthralgia and rash were significantly associated with NS1 positive status with p=0.003. Abdominal pain and headache showed non-significant association with NS1 status with p=0.07 and 0.14 respectively. Retro-orbital pain and myalgia showed significant association with NS1 positive status with p=0.01. These results were consistent with the findings of Chakravarti A et al[7] in 2012 and Karoli R et al[8] in 2012 where clinical features of dengue fever assessed with NS1 antigen similar to the present study were reported by the authors in their respective studies.

Concerning blood counts, in TLC, leucocytosis, leucopenia, and normal counts were seen in 6% (n=2), 16% (n=8), and 78% (n=39) subjects respectively with mean TLC as 5500±2489.64. Normal platelet counts and thrombocytopenia were seen in 14% (n=7) and 86% (n=43) subjects respectively with a platelet count of 37120.55±32304.97. PCV% of >50 and <50 was seen in12% (n=6) and 88% (n=44) subjects with mean PCV of 42.42±8.32%. NS1 negative and positive status was seen in 32% (n=16) and 68% (n=34) subjects respectively. IgM negative status was seen in 32 NS1 positive and 2 NS1 negative subjects. IgM positive status was seen in 35 NS1 positive and 15 NS1 negative subjects respectively. The sensitivity, specificity, positive predictive value, and negative predictive values were 92.84%, and 90%. 95.57%, and 84.36%

respectively with p<0.01. These findings were in agreement with the results of Libraty DH et al[9] in 2002 and Kulkarni RD et al[10] in 2011 where authors reported similar associations in their studies of NS1 antigen and blood counts, IgM status, specificity, and sensitivity as seen in the present study.

The study results showed that for the correlation of the NS1 test to clinical findings, the statistically non-significant association was seen in CNS, hypotension, and pulse rate with p=0.13, 0.14, and 0.15 respectively. A significant association of the NS1 test to abdominal pain, edema, and icterus with p=0.003, 0.03, and 0.01 respectively. For the correlation of blood investigations to the NS1 antigen test in study subjects, TLC showed a statistically non-significant association with NS1 antigen with p=0.12. A similar non-significant association was seen in NS1 antigen and PCV (%) with p=0.08. However, a significant association was seen in platelet counts and NS1 antigen test with p=0.002. These results were in line with the findings of Datta S et al[11] in 2010 and Dash PK[12] in 2004 where correlation of NS1 test to clinical findings and blood investigations similar to the present study was reported by the authors in their respective studies.

It was seen that for the assessment and correlation of ECG and NS1 findings in study subjects. Sinus bradycardia was seen in 35.29% (n=12) NS1 positive subjects and 18.75% (n=3) NS1 negative subjects respectively. Normal ECG findings were seen in 64.70% (n=22) subjects with NS1 positive status and 81.25% (n=13) subjects with NS1 negative subjects. The difference was statistically non-significant with p=0.15. These findings correlated with the studies of Neeraja M et al[13] in 2006 and Shu PY[14] in 2004 where a similar correlation of ECG and NS1 findings was reported by the authors as seen in the results of the present study.

CONCLUSIONS

The present study considering its limitations concludes that dengue infection that poses a major healthcare issue can be diagnosed early with clinical features as SGOT>SGPT, thrombocytopenia, bleeding manifestations, myalgia, and retro-orbital pain which is supported by detecting NS1 antigen.

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S. No	Characteristics	Number (n=50)	Percentage (%)
1.	**Gender**		
a)	Males	31	62
b)	Females	19	38
2.	**Age range (years)**		
a)	<30	30	60
b)	31-40	8	16
c)	41-50	5	10
d)	51-60	6	12
e)	>60	1	2

Table 1: Demographic data of study participants

S. No	Complaints	NSI positive		NSI negative		p-value
		n=34	%	n=16	%	
1.	**Diarrhea**	1	100	0	0	0.34
		33	67.34	16	32.65	

2.	**Vomiting**	8	80	2	20	0.07
		26	65	14	35	
3.	**Bleeding**	11	100	0	0	**<0.001**
		23	58.97	16	41.02	
4.	**Arthralgia**	13	81.25	3	18.75	**0.003**
		21	61.76	13	38.24	
5.	**Rash**	15	83.33	3	16.6	**0.003**
		19	59.37	13	40.62	
6.	**Abdomen pain**	20	80	5	20	0.07
		14	56	11	44	
7.	**Retro-orbital pain**	24	75	8	25	**0.01**
		10	55.5	8	44.4	
8.	**Myalgia**	27	75	9	25	**0.01**
		7	50	7	50	
9.	**Headache**	27	64.28	15	35.71	0.14
		7	87.5	1	12.5	

Table 2: Clinical features of dengue fever assessed with NS1 antigen in subjects assessed till 7 days of fever onset

S. No	Variables	Number (n=50)	Percentage (%)
1.	**TLC (total leukocyte counts)**		
a)	Leucocytosis	3	6
b)	Leucopenia	8	16
c)	Normal	39	78
d)	Mean± S. D	5500±2489.64	
2.	**Platelet counts**		
a)	Normal	7	14
b)	Thrombocytopenia	43	86
c)	Mean± S. D	37120.55±32304.97	
3.	**PCV (%)**		
a)	>50	6	12
b)	<50	44	88
c)	Mean± S. D	42.42±8.32	

Table 3: Blood counts in study subjects

S. No	Findings	Number (n=50)	Percentage (%)
1.	**NS1 negative**	16	32
2.	**NS1 positive**	34	68
3.	**IgM**	**NS1 Positive**	**NS1 Negative**
a)	Negative	33	2
b)	Positive	2	13
4.	Total	35	15
5.	**Sensitivity (%)**	92.84	
6.	**Specificity (%)**	90	

7.	**Positive predictive value (%)**	95.57
8.	**Negative predictive value (%)**	84.36

Table 4: NS1 findings, diagnostic efficacy of NS1 compared to IgM ELISA after 7 days

S. No	Clinical findings	NSI positive		NSI negative		p-value
		n=34	%	n=16	%	
1.	**CNS**					0.13
a)	Altered sensorium	2	100	0	0	
b)	Normal	32	66.6	16	33.3	
2.	**Pain abdomen**					**0.003**
a)	Normal	14	56	11	44	
b)	Tender	20	80	5	20	
3.	**Oedema**					
a)	Present	5	100	0	0	**0.03**
b)	Absent	29	64.4	16	35.5	
4.	**Icterus**					**0.01**
a)	Present	8	88.8	1	11.1	
b)	Absent	26	63.41	15	36.58	
5.	**Hypotension**					0.14
a)	Present	2	100	0	0	
b)	Absent	32	66.6	16	33.3	
6.	**Pulse rate**					0.15
a)	<60	12	77.42	3	22.58	
b)	>60	22	64.70	12	35.29	

Table 5: Correlation of NS1 to clinical findings

S. No	Blood findings	NSI positive		NSI negative		p-value
		n=34	%	n=16	%	
1.	**TLC (total leukocyte counts)**					0.12
a)	Leucocytosis	1	33.3	2	66.6	
b)	Leucopenia	6	85.71	1	14.28	
c)	Normal	27	67.5	13	32.5	
2.	**Platelet counts**					**0.002**
a)	Normal	2	33.3	4	66.6	
b)	Thrombocytopenia	32	72.7	12	27.2	
3.	**PCV (%)**					0.08
a)	>50	29	65.90	15	34.09	
b)	<50	5	83.3	1	16.6	

Table 6: Correlation of NS1 to blood investigations

S. No	Findings	NSI positive		NSI negative		p-value
		n=34	%	n=16	%	
1.	**Sinus bradycardia**	12	35.29	3	18.75	0.15

2.	**Normal**	22	64.70	13	81.25	

Table 7: ECG and NS1 findings in study subjects