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Original research article

A study on early diagnosis of dengue by clinical features and serological markers in a tertiary care hospital

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Abstract

Background: Arboviruses pose a major threat to the public's health. In tropical and subtropical areas of the world, they are usually connected with epidemics that have significant negative economic and social effects. High rates of morbidity and mortality are caused by the dengue virus (DENV), which is transmitted to humans by mosquitoes of the Aedes family.[1]

Objectives

1. To find out the role of NS1 antigen for early detection of dengue virus infection versus IgM ELISA.
2. To study the clinical features in early stage of dengue infection.

Material & Methods

Study Design: A prospective hospital based observational study.

Study area: Department of General Medicine, Government Medical College, Kadapa, Andhra Pradesh.

Study Period: 1 year.

Study population: The study was comprised of adult patients presenting with clinical features suggestive of dengue infection.

Sample size: A total of 100 adult patients presenting with clinical features suggestive of dengue infection were selected for the study.

Sampling method: Simple random technique.

Study tools and data collection procedure: Patients were interviewed for demographic data such as age sex and occupation were noted. Histories of similar complaints in past and current treatment were noted. Patients were subjected to a thorough physical examination, vitals (pulse rate, blood pressure and respiratory rate) and other clinical signs and symptoms of dengue fever. Systemic examination was carried out. These findings were recorded on a predesigned and pretested proforma.

Results: In the present study 70 patients tested positive for IgM ELISA. Of these, 65 were positive for NS1 antigen test while five were negative. The sensitivity of NS1 in predicting dengue infection compared to IgM ELISA was 92.86% and specificity was 90% with 95.59% positive predictive value and 84.38% negative predictive value ($p<0.001$).

Conclusion: Thus we conclude that dengue infection, which poses a serious public health problem, can be diagnosed early with the help of clinical features like retro-orbital pain, myalgia, bleeding manifestations, thrombocytopenia, SGOT greater than SGPT that is supported by detection of NS1 antigen.

Keywords: Dengue virus, rapid diagnostic test, thrombocytopenia

Introduction

Arboviruses pose a major threat to the public's health. In tropical and subtropical areas of the world, they are usually connected with epidemics that have significant negative economic and social effects. High rates of morbidity and mortality are caused by the dengue virus (DENV), which is transmitted to humans by mosquitoes of the Aedes family [1].

The main virus transmitted to humans by arthropods is dengue fever. More than 50 million infections are thought to occur annually, with 500,000 hospitalisations for dengue hemorrhagic fever, mostly among youngsters and a case fatality rate that exceeds 5% in some regions. Nearly 120 nations have been affected by [2-5] DF epidemics and several of them have a high incidence [6].

The family Flaviviridae and the genus Flavivirus both contain the dengue virus (DENV). Based on the size of its single-stranded positive sense RNA, which is roughly 11 kb, the virus is categorised into four serotypes called DENV-1, DENV-2, DENV-3 and DENV-4 properties that are antigenic. The DENV genome consists of 10 open reading frames (ORFs), each of which is translated into a polyprotein that is then broken down by the host cell's signal peptidase into three structural proteins (C, E, and pr M) and

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seven nonstructural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5). Clinical symptoms of infection by any of the aforementioned serotypes range, are generic, and are benign until more severe phases, which can occasionally have deadly repercussions in the form of dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) [7].

Clinicians cannot determine which patients will develop severe disease during the early febrile stage, which is characterised by symptoms including fever, malaise, headache, bodily aches, and rash. Later, during effervescence, symptoms such as bleeding, thrombocytopenia of <100,000 platelets mm^{-3}, ascites, pleural effusion, increase in hematocrit of >20% and clinical warning signs, such as severe and continuous abdominal pain, restlessness and/or somnolence, persistent vomiting and a sudden reduction in temperature associated with profuse perspiration, and sometimes fainting, can be indicative of plasma extravasation and the imminence of shock. Numerous organ failure, disseminated intravascular coagulation, severe haemorrhage and respiratory failure brought on by non-cardiogenic pulmonary oedema are some consequences [8-11].

Detection of IgM or IgG antibodies is the standard for serologically confirming a dengue infection. The presence of IgM or high levels of IgG in acute serum collected from a suspected dengue case suggests a probable dengue infection [8-9]. The confirmation of the dengue infection is done by virus isolation, genome detection, antigen detection and IgM or IgG seroconversion. However, the probable dengue infection is done by IgM positive and elevated IgG titre (that is, 1,280 or greater by haemagglutination inhibition test).

Recently, an up-to-date test for early diagnosis of dengue infection is dengue NS1 antigen detection. NS1 is a glycoprotein produced by all flaviviruses and is essential for viral replication and viability. Because this protein is secreted into the bloodstream, many tests have been developed to diagnose DENV infections using NS1. These tests include antigen- capture ELISA, lateral flow antigen detection and measurement of NS1-specific IgM and IgG responses. NS1 antigen detection kits are now commercially available [12].

Evaluation of the NS1 assay indicated moderately high sensitivity and very high specificity to dengue infection. Also NS1 test is cost effective and provides results earlier. However, NS1 being the newer test very few studies have reported the role of NS1 antigen detection in the early diagnosis of dengue virus infection. Hence the present study was undertaken to find out the role of NS1 antigen for early detection of dengue virus infection versus ELISA and to study the clinical features in early stage of dengue infection.

Objectives

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Sample size: A total of 100 adult patients presenting with clinical features suggestive of dengue infection were selected for the study.

Sampling method: Simple random technique.

Inclusion criteria

- Age more than 12 years.
- History of documented fever of more than 380 C of less than seven days plus.
- Two or more signs and symptoms from the following.
  - Headache
  - Retro-orbital pain
  - Myalgia
  - Arthralgia
  - Rash
  - Hypotension
  - Bleeding manifestations

Exclusion criteria: Localised source of infection.

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Study tools and data collection procedure

Patients were interviewed for demographic data such as age sex and occupation were noted. Histories of similar complaints in past and current treatment were noted. Patients were subjected to a thorough physical examination, vitals (pulse rate, blood pressure and respiratory rate) and other clinical signs and symptoms of dengue fever. Systemic examination was carried out. These findings were recorded on a predesigned and pretested proforma.

Investigations

The selected patients underwent the following investigations.

- Complete blood count.
- Renal function tests.
- Liver function tests.
- NS1 antigen testing for dengue.
- Dengue IgM ELISA after 7 days.
- Electrocardiography.

Under strict aseptic precautions, 3 mL blood was drawn by venipuncture. NS1 antigen testing for dengue was done using the Dengue NS1Ag+Ab Combo SD BIOLINE Dengue Duo kit manufactured by Standard Diagnostics Inc. It is a rapid, an *in-vitro* immunochromatographic, one step assay designed to detect dengue virus NS1 antigen. The sensitivity and specificity of the kit was confirmed with IgM ELISA done later in the course of illness.

Statistical analysis

The data obtained was coded and entered into Worksheet. The categorical data was expressed as rates, ratios and proportions and comparison was done using chi-square test. The continuous data was expressed as mean ± standard deviation (SD). The diagnostic accuracy of NS1 antigen testing, in predicting dengue infection was determined by sensitivity, specificity, positive predictive value and negative predictive value. Kappa agreement was used to correlate the agreements. A probability value ('p' value) of less than or equal to 0.05 was considered as statistically significant.

Observations & Results

Table 1: Sex distribution

Sex	Number	Percentage
Male	62	62.00
Female	38	38.00
Total	100	100.00

In the present study 62% of patients were males and 38% of females. The male to female ratio was 1.63:1.

Table 2: Age distribution

Age group	Distribution (n=100)	
(Years)	Number	Percentage
< 30	60	60.00
30 to 40	15	15.00
41 to 50	10	10.00
51 to 60	12	12.00
> 60	3	3.00
Total	100	100.00

In this study most of the patients (60%) were aged less than 30 years followed by 30 to 40 years (15%), 51 to 60 years (12%) and 41 to 50 years (10%). The mean age of the study population was 32.48 ± 13.87 years.

In the present study all the patients presented with fever (100%). Headache, myalgia, retro-orbital pain and pain abdomen was reported by 82%, 72%, 62% and 49% of patients respectively. Bleeding manifestations and rashes were present in 22% and 36% of the patients respectively.

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Table 3: Clinical features associated with dengue diagnosis using NS1 antigen test in patients evaluated up to 7 days of fever onset

Presenting complaints	NS1 positive		NS1 negative		p value
	Number	Percentage	Number	Percentag	
Headache	53	64.63	29	35.37	0.124
	15	83.33	3	16.67	
Myalgia	54	75.00	18	25.00	0.016
	14	50.00	14	50.00	
Retro orbital pain	47	75.81	15	24.19	0.016
	21	55.26	17	44.74	
Pain abdomen	40	81.63	9	18.37	0.069
	28	54.90	23	45.10	
Rash	31	86.11	5	13.89	0.004
	37	57.81	27	42.19	
Arthralgia	25	80.65	6	19.35	0.004
	43	62.32	26	37.68	
Bleeding manifestation	22	100.00	0	0.00	<0.001
	46	58.97	32	41.03	
Vomiting	17	85.00	3	15.00	0.068
	51	63.75	29	36.25	
Diarrhoea	2	100.00	0	0.00	0.327
	66	67.35	32	32.65	

In this study we see that myalgia, retro orbital pain, arthralgia, rash and bleeding manifestations were significantly associated in patients who were NS1 positive.

In the present study, the clinical examination revealed, hypotension in 4% of patients. Icterus and oedema was observed in 16% and 9% of patients respectively. Bradycardia was present in 31% of the patients. In this study, systemic examination revealed, decreased AE in bilateral bases and bilateral CC in 16% and 5% of patients in respiratory system. Abdominal tenderness was seen in 49% of patients and altered sensorium was present in 5% of patients.

Table 4: Complete blood count

Variables	Findings (n=100)		
		Number	Percent
Packed cell volume (%)	> 50	11	11.00
	< 50	89	89.00
	Total	**100**	**100.00**
	Mean ± SD	**42.40**	**8.30**
Platelet count (/mm^3)	Thrombocytopenia	87	87.00
	Normal	13	13.00
	Total	**100**	**100.00**
	Mean ± SD	**37120.57**	**32304.99**
Total leukocyte count			
Normal		78	78.00
Leucopenia		16	16.00
Leucocytosis		6	6.00
Total		**100**	**100.00**
Mean ± SD		**5500.00**	**2489.66**

The haematocrit was increased in 11% of the patients while thrombocytopenia was present in 87% of the patients and leucopenia in 16% with mean values being 42.40 ± 8.30 cmm, 37120.57 ± 32304.99/mm3 and 5500 ± 2489.66 /mm3 respectively.

In this study, total bilirubin was raised in 25% of patients and the mean total bilirubin was found to be 1.04 ± 1.17 mg/dL. The SGOT and SGPT was abnormal in 76% and 51% of patients with mean values being 122.04 ± 172.27 and 91.81 ± 123.60 IU/L respectively. The alkaline phosphatase was raised in 43% of patients with mean values being 144.39 ± 91.99. In this study ecg findings revealed sinus bradycardia in 31% of patients.

Table 5: NS1 test findings

Distribution (n=100)		
Findings		
	Number	**Percentage**
NS1 positive	68	68.00
NS1 Negative	32	32.00
Total	**100**	**100.00**

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In this study NS1 test was positive for dengue infection 68% of patients.

Table 6: Diagnostic accuracy of NS1 in comparison to IgM ELISA after seven days

	IgM		
NS1 test			**Total**
	Positive	**Negative**	
Positive	65	3	68
Negative	5	27	32
Total	70	30	100

P<0.0001
Kappa= 0.813
SE of kappa = 0.063
95% confidence interval: From 0.689 to 0.937
The strength of agreement is considered to be 'very good'.

Sensitivity (%)	**Specificity (%)**	**PPV (%)**	**NPV (%)**
92.86%	90%	95.59%	84.38%

In the present study 70 patients tested positive for IgM ELISA. Of these, 65 were positive for NS1 antigen test while five were negative. The sensitivity of NS1 in predicting dengue infection compared to IgM ELISA was 92.86% and specificity was 90% with 95.59% positive predictive value and 84.38% negative predictive value (p<0.001).

Table 7: Association of clinical signs with NS1 antigen test results

NS1 findings						
Variables		**Positive (n=68)**		**Negative (n=32)**		**p value**
		No	**%**	**No**	**%**	
Pulse rate	< 60 /min	24	77.42	7	22.58	0.175
	> 60 /min	44	63.77	25	36.23	
Hypotension	Present	4	100.00	0	0.00	0.161
	Absent	64	66.67	32	33.33	
Icterus	Present	15	93.75	1	6.25	0.016
	Absent	53	63.10	31	36.90	
Oedema	Present	9	100.00	0	0.00	0.031
	Absent	59	64.84	32	35.16	
RS	Normal	51	62.96	30	37.04	0.073
	Air entry ↓ B/L	13	86.67	2	13.33	
	Bases B/L Coarse crepitation	4	100.00	0	0.00	
PA	Normal	28	54.90	23	45.10	0.004
	Tenderness present	40	81.63	9	18.37	
CNS	Normal	63	66.32	32	33.68	0.116
	Altered sensorium	5	100.00	0	0.00	

Icterus and oedema were present significantly more in patients who tested positive for NS1 antigen (p < .0.016 and p< .0.031) respectively. Similarly, respiratory findings and abdominal tenderness were significantly more in NS1 positive patients (p <0.05).

Table 8: Association of CBC profile with NS1 antigen test results

NS1 findings						
Variables		**Positive (n=68)**		**Negative (n=32)**		**p value**
		No	**%**	**No**	**%**	
PCV (%)	< 50	58	65.17	31	34.83	0.084
	> 50	10	90.91	1	9.09	
Platelet						
	Normal	4	30.77	9	69.23	0.002
	Thrombocytopenia	64	73.56	23	26.44	
TLC						
	Leucopenia	13	81.25	3	18.75	0.100
	Normal	53	67.95	25	32.05	
	Leucocytosis	2	33.33	4	66.67	

In this study significantly higher number of patients with decreased platelet count (73.56%) had positive NS1 antigen test (p=0.002).

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Table 9: NS1 and ECG findings

Findings	NS1 Positive (n=68)		NS1 Negative (n=32)	
	No	%	No	%
Normal	44	63.77	25	78.12
Sinus bradycardia	24	35.23	7	22.58
p=0.176				

In this study ECG revealed sinus bradycardia in 35.23% of patients with positive NS1 antigen test while 63.77% of patients had normal ECG who were positive on NS1 antigen test. This difference was statistically not significant (p=0.176).

Discussion

In the present study slight male preponderance was seen that is, 62% of patients were males and the male to female ratio was 1.63:1. These findings were comparable with a study conducted by Agarwal *et al.* [13] in which male to female ratio was 1.9:1. Another study conducted by Sharma *et al.* [14] showed that male to female ratio was 3:1. The incidence of dengue is equal in males and females. However, fewer cases of dengue hemorrhagic fever and dengue shock syndrome have been reported in men than in women.

In this study most of the patients (60%) were aged less than 30 years followed by 30 to 40 years (15%), 51 to 60 years (12%) and 41 to 50 years (10%). The mean age of the study population was 32.48 ± 13.87 years. Dengue affects people of all ages. A retrospective study to review the changing epidemiology of the dengue between the years 2002 and 2008 by Chakravarthy A *et al*., reported presence of dengue in all the age groups of study population and noted predominance of adult population [15].

In the present study 100% of the patients presented with fever, followed by headache in 82%, myalgia in 72%, retro-orbital pain in 62% and pain abdomen in 49% of patients. On clinical examination, icterus and edema was observed in 16% and 9% of patients that were statistically significant in NS1 positive patients (p 0.016 and p 0.031) respectively. Hypotension was seen in 4% of patients all of whom were NS1 positive. Abdominal tenderness was present in 49% of patients, arthralgia in 31%, rashes in 36% and neurological involvement was present in 5% of patients.

In our study bleeding manifestations were present in 22% of the patients of which majority presented with malena (8%). Bleeding manifestations were significantly more in NS1 positive patients (p<0.001).

In a study by Shivbalan S *et al.* [16] during 2004 on the predictors of spontaneous bleeding in dengue, a platelet count of less than 50,000 was found to be significantly associated with increased risk of bleeding. The other associated predictors of bleeding in the study conducted were prolonged PT, raised AST/ALT and haemo concentration. Serum sodium and serum potassium were found to be abnormal in 5% and 3% of patients respectively. The ECG findings revealed sinus bradycardia in 31% of patients. In our study all patients in altered sensorium (5%) were positive for NS1 antigen. This study showed 16% of the patients had air entry decreased bilateral bases and 5% had coarse crepitations bilaterally.

More recently in 2012 Karoli R *et al.* [17] in their cross-sectional study at Lucknow during the monsoon and post-monsoon seasons in the year 2010 on 356 patients with suspected dengue fever found 138 (39%) had serologically confirmed dengue infection. Out of this Ninety-six (70%) patients had classical dengue fever while 42 (30%) had dengue hemorrhagic fever. The most common symptoms were headache (76%), abdominal pain (63%), vomiting (58%), rash (26%), and cutaneous hypersensitivity (16%). Hemorrhagic manifestations were present in 55 (40%) patients. Notably, 14% of patients had neurological involvement and 4% had acute hepatic failure. Study concluded that, dengue infection had varied and multi-systemic manifestations that can go unrecognized.

In this study NS1 test was positive for dengue infection in 68% of patients and 70% of patients were positive for dengue infection on IgM. Of these, 65 were positive for dengue infection on NS1 while five were negative. The sensitivity of NS1 in predicting dengue infection compared to IgM was 92.86% and specificity was 90% with 95.59% positive predictive value and 84.38% negative predictive value (p<0.001). The strength of agreement was considered to be 'very good' based on Kappa statistics (Kappa 0.813; SE of kappa = 0.063; 95% confidence interval: From 0.689 to 0.937).

The NS1 antigen test for the diagnosis of acute dengue infection using admission samples had demonstrated considerable variation in sensitivity (49.8%-8.7%) but the specificities reported were more consistent with all being >90% [18].

Libraty *et al*. observed that a very high concentration of NS1 antigen within 72 hours of illness identified patients at risk of developing DHF [19]. A study by Datta S *et al.* [20] in New Delhi to evaluate the efficacy of NS1 antigen (Ag) assay as an early marker for dengue virus (DV) infection concluded that, NS1 Ag assay holds promise in early diagnosis of dengue infection. When used in combination with MAC-ELISA on a single sample it significantly improves the diagnostic algorithm without the requirement of paired sera.

A study by Singh MP *et al.* [21], in Chandigarh compared IgM antibody detection with NS1 antigen for the diagnosis of acute dengue in 87 samples. NS1 antigen could be detected with good sensitivity (71-100%) till day 3 of fever, whereas IgM had a sensitivity of 0% to 50% at this time. On day 4 of illness,

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both the tests had comparative sensitivity. Beyond day 4, IgM antibody detection was superior to NS1. Both these diagnostic modalities were also compared with RT-PCR in 40 acute samples. NS1 detected additional 15 samples, which were missed by PCR. Study concluded that, NS1 antigen is an early diagnostic marker that is feasible in a routine diagnostic laboratory.

In this study significantly higher number of patients with decreased platelet count (73.56%) had positive NS1 antigen test for dengue infection (p=0.012). The findings of the present study were similar to the study done by Kulkarni RD *et al*. [21] Overall the present study showed the usefulness of NS1 antigen test which is an excellent tool in addressing potentially fatal, epidemic prone dengue infection based on its easy and fast application compared to immunochromatography based dengue serology tests. Similarly, clinical features like retro orbital pain, myalgia, arthralgia, rash, bleeding manifestations along with laboratory findings like thrombocytopenia, SGOT>SGPT strongly support the diagnosis of dengue fever.

Conclusion

In our study NS1 RDT showed promising results with sensitivity and specificity of 92.86% and 90% respectively when compared with IgM ELISA done later in the course of illness. Considering only clinical features, in our study we found that presence of retro orbital pain, myalgia, bleeding manifestations, rashes and arthralgia were significantly (p value <0.05) more in patients who were NS1 positive. Thus we conclude that dengue infection, which poses a serious public health problem, can be diagnosed early with the help of clinical features like retro-orbital pain, myalgia, bleeding manifestations, thrombocytopenia, SGOT greater than SGPT that is supported by detection of NS1 antigen.

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