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Clinico-Microbiological Study of Dengue Virus Infection in a Tertiary Care Hospital

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A B S T R A C T

Dengue fever is an arthropod borne viral disease present as either a non-specific illness, dengue fever (DF) or DHF, DSS leading to hospitalization or death. In routine laboratories diagnosis is mainly based on the detection of dengue antigens (NS1) or antibodies (IgM and IgG) by ELISA kits. Objectives of the study are to find out the seropositivity of dengue virus infection in patients admitted in a tertiary care hospital and to study the clinical presentation of dengue in serologically diagnosed cases. The study was conducted Department of Microbiology, GMC Aurangabad. Samples from Clinically suspected cases of Dengue are collected along with clinical profile and Patients were divided into two groups, Group A containing patients having fever history of 5 days or less than 5 days and Group B containing patients having fever history of more than 5 days. Serum samples from both Group A and Group B were tested by Rapid Immunochromatographic Card test (RICT). In addition to RICT, Serum samples from Group A were subjected to NS1 ELISA and serum samples from Group B subjected to IgM Capture ELISA. Out of 390 clinically suspected dengue cases 138 cases (35.38%) were serologically positive by RICT and or ELISA.111 cases were positive by RICT. In Group A (180 samples), 55 samples were positive by NS1 ELISA and In Group B (210 samples), 83 samples were positive by IgM ELISA. A total of 55 cases i.e. 30.55% (55/180) were positive for NS1 ELISA as compared to 42 Cases *i.e.* 23.34% (55/180) by RICT for NS1 Ag (During 1-5 days of Fever) in Group A. A total of 83 cases *i.e.* 39.52% (83/210) were positive for IgM ELISA as compared to 66 cases *i.e.* 31.42% (83/210) by RICT for IgM Ab (During 6-10 days of fever) in Group B. NS1 antigen was detectable in patient's sera from day one of fever, IgM antibodies from day 3 onwards. Out of 138 dengue positive cases, majority of the cases *i.e.* 84 (60.87%) were having dengue fever without warning signs, followed by 39 cases (28.26%) cases of dengue with warning signs and 15 cases (10.86%) had severe dengue. Fever was most common clinical presentation followed by Headache, bodyache, nausea, vomiting, joint pain, abdominal pain, retro-orbital pain and rash, hemorrhagic manifestations. Hepatomegaly was the commonest clinical sign followed by ascites, pleural effusion and Splenomegaly. Death occurred in 4 (2.89%) cases. RICT provide the useful aids in screening and ELISA should be considered as the confirmatory test in diagnosis of dengue.NS1 ELISA has promising results in early diagnosis of dengue and when used in combination with IgM Capture ELISA it increases the diagnostic efficiency. Hence involvement of good laboratories for early and prompt diagnosis of dengue infection, coupled with vector control programmes and inducing awareness among the public, is needed in combating future epidemics of dengue.

Introduction

Dengue fever is an important arthropod (mosquito) borne viral disease of tropics and subtropics affecting urban and periurban areas. It is a self limiting disease transmitted by bite of an infected female *Aedes* mosquito (Manohar *et al.,* 2015). According to the World Health Organization (WHO) estimates, the incidence of dengue has increased by a factor of 30 over the last 50 years (Dussart *et al.,* 2006). 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 dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) causing around 30000 deaths (Patil *et al.,* 2014). Dengue is now endemic in more than 100 countries (Dussart *et al.,* 2006).

In several Asian countries, dengue is the leading cause of hospitalization and death in children.

Hence, there is a need for diagnostics, prophylactics and therapeutics to manage it (Shrivastava *et al.,* 2011).

Infection with a dengue virus may be clinically inapparent or may present as a non-specific febrile illness, classic dengue fever or DHF. Classic dengue fever is characterized by fever, malaise, headache, arthralgia, myalgia and rash. DHF is characterized by onset of plasma leakage, marked thrombocytopenia and bleeding diathesis. Severe plasma leakage can lead to DSS with mortality rate for untreated patients being in excess of 10% (Vaughn *et al.,* 1998).

Primary DENV infections present as either a non-specific illness or dengue fever (DF). Secondary infection with a serotype different from that causing primary infection may lead to DHF or DSS (Moorthy *et al.,* 2009).

Several approaches have been applied for laboratory diagnosis of dengue virus infections.

These methods include detection of the virus (by cell culture or immunofluorescence), detection of virus antigen (Ag) (by enzyme-linked immunosorbent assay [ELISA]), detection of anti-dengue virus antibody (by hemagglutination inhibition [HI], complement fixation test [CF], neutralization test, or ELISA), and detection of virus nucleic acids (by reverse transcription [RT]-PCR or real-time RT-PCR).(Wang SM *et al.,*2010). Thus, in a majority of cases the only feasible diagnosis is based on the detection of dengue antigens or antibodies (Shrivastava *et al.,* 2011).

Dengue IgM and IgG ELISA kits are widely used for diagnosis of dengue infection in routine laboratories. However, there are variations in detection limit during acute phase of the disease (Ahmed *et al.,* 2014). An ELISA specific to dengue virus NS1 protein has been developed for the detection of dengue NS1 antigen during the acute phase of disease in patients experiencing primary and secondary infections (Shrivastava *et al.,* 2011).

The recently introduced dengue rapid kits rapid immunochromatographic card test (RICT) in Indian market are combination packs, which detect circulating NS1 antigen, a highly conserved viral protein and IgM and IgG (Stephen *et al.,* 2014)

Currently, no effective antiviral agents to treat dengue infection are available. The management of dengue virus infection is essentially supportive and symptomatic. With limited therapeutic strategies and the current lack of a vaccine, A rapid and accurate diagnosis of dengue in the acute phase of illness is important for initiation of therapy & forecasting an early warning of an epidemic

and in undertaking effective vector control measures are essential to reduce dengue-related mortality and morbidity (Shrivastava *et al.,* 2014; Mahapatra *et al.,* 2014; Gupta *et al.,* 2014). The present study was to evaluate utility of serodiagnosis of dengue virus infection and to study clinical profile in serologically diagnosed cases. It will help the clinicians in early diagnosis, proper management and effective treatment which may minimize the complications.

To find out the seropositivity of dengue virus infection in patients admitted in a tertiary care hospital.

To study the clinical presentation of dengue in serologically diagnosed cases.

Materials and Methods

The present study was conducted in a tertiary care hospital in the Department of Microbiology after obtaining approval from the institutional Ethical Committee.

The study was carried out over a period of one & half years from January 2014 to June 2015.

Inclusion criteria

The clinically suspected patients of dengue virus infection admitted in a tertiary care hospital

The clinically suspected patients of dengue virus infection presented within 1-10days of fever

Those ready to give informed written consent

Exclusion criteria

Patients with history of fever which the clinician doesn't correlate with dengue virus infection were excluded.

Fever with any other proven microbial illness (bacterial, viral, parasitic infection)

The clinically suspected patients of dengue virus infection presented with fever more than 10 days.

Those not ready to give informed written consent.

Type of study

Prospective, observational, descriptive study

Sample size

The clinically suspected patients of dengue virus infection admitted in tertiary care hospital during study period from January 2014 to June 2015

Clinical data

After informed consent, the clinical data of suspected patients was collected as per Performa.

Sample collection

By using universal aseptic precautions 2-3ml of blood of the patient clinically suspected of dengue fever were collected by the referral departments in plain bulb

Laboratory methods

Blood in plain bulb sent to microbiology laboratory was centrifuged at 3000 rpm for 5 min and serum was separated.

Patients were divided into two groups,

Group A having fever history of 5 days or less than 5 days.

Group B having fever history of more than 5 days.

All Serum samples of group A and Group B were tested by rapid immunochromatographic card test (RICT) according to manufacturer's guidelines (dengue NS1 Ag and Ab Combi Card test, J Mitra and Co. Pvt. Ltd., New Delhi India). Then all samples in group A were subjected to NS1 ELISA according to manufacturer's guidelines. (DENGUE NS1 Ag MICROLISA manufactured by J.Mitra & Co. Pvt. Ltd New Delhi, India)

All samples in group B were subjected to IgM Capture ELISA according to manufacturer's guidelines (manufactured by National Institute of virology Pune, India).

Based on clinical and laboratory findings dengue positive cases were classified into

Dengue without warning signs

Dengue with warning signs

Severe dengue

Results and Discussion

The present study was carried out in a tertiary care hospital during one & half year period from January 2014 to June 2015. Patients were divided into two groups, Group A containing 180 patients having fever history of 5 days or less than 5 days and Group B containing 210 patients having fever history of more than 5 days. Serum samples from both Group A and Group B (180+210=390) were tested for the presence of NS1 antigen, IgM and IgG antibodies by Rapid Immunochromatographic Card test (RICT). In addition to RICT, Serum samples from Group A (180 Samples) were subjected to NS1 ELISA and serum samples from Group B (210 samples) subjected to IgM Capture ELISA.

Out of 390 clinically suspected dengue cases 138 cases (35.38%) were serologically positive shown in Table 1. In Group A (180 samples), 55 samples were positive by RICT &/or NS1 ELISA and In Group B (210 samples), 83 samples were positive by RICT &/or IgM ELISA. Out of 390 clinically suspected dengue cases (Group A and Group B) 138 cases (35.38%) were positive shown in Table 2.

In the present study, fever was present in all 138 (100%) of cases. Headache was the second most common clinical presentation which was observed in 107 (77.53%) cases followed by bodyache in 92 (66.67%) cases, nausea and vomiting in 66 (47.82%) cases, joint pain in 63 (45.65%) cases, abdominal pain in 53 (38.40%) cases, retro-orbital pain in 53 (38.40%) cases, rash in 30 (21.73%) cases, hemorrhagic manifestations in 21 (15.21%) cases shown in Graph 1.

Hepatomegaly was the commonest clinical sign, which was found in 21 cases (15.21%) followed by ascites in 16 cases (11.59%). pleural effusion in 14 (10.14%) and Splenomegaly in 11 cases (7.97%) shown in Table 3.

Out of 138 dengue cases death occurred in 4 (2.89%) cases.

Majority of the cases i.e. 84 (60.87%) were having dengue fever without warning signs, followed by 39 cases (28.26%) cases of dengue with warning signs and 15 cases (10.86%) had severe dengue shown in Table 4. 111 cases were positive by RICT and of which majority cases were only IgM antibody positive i.e. 48 (43.24%) cases followed by only NS1 antigen positive cases i.e. 27 (24.32%) shown in Table 5.

RICT detects either NS1 Ag, IgM or IgG antibodies. Samples positive for only NS1 Ag or only IgM Ab or NS1 Ag and IgM Ab were classified as primary dengue infections.

Samples positive for IgG Ab, or NS1 Ag and IgG Ab, or IgM and IgG Ab or NS1 Ag, IgM Ab and IgG Ab were classified as secondary dengue infections. Of the 111 positive cases by RICT, 89 cases (80.18%; 89/111) were classified as primary dengue and remaining 22 (19.82%; 22/111) cases as secondary dengue infection shown in Table 6.

The results shows NS1 antigen was detectable in patient's sera from day one of fever, IgM antibodies from day 3 onwards and NS1 Ag was detected in patients sera till the 8th day of fever by RICT shown in Table 7.

From the table it is evident that RICT NS1 Ag positivity was seen more in Group A 42(84%) than in Group B 8(16%) and RICT IgM Ab positivity was seen more in Group B 66(88%) than in Group A 9(12%).

This has been found to be statistically significant ($p<0.0001$) as shown in Table 8. A total of 55 cases *i.e.* 30.55% were positive for NS1 ELISA as compared to 42 Cases *i.e.* 23.34% by RICT for NS1 Ag (During 1-5 days of Fever) in Group A as shown in Table 9.

A total of 83 cases i.e. 39.52% were positive for IgM ELISA as compared to 66 cases i.e. 31.42% by RICT for IgM Ab (During 6-10 days of fever) in Group B as shown in Table 10.

The results table shows NS1 ELISA Positivity for NS1 Ag was 30.55% and IgM ELISA Positivity for IgM Ab was 39.52% as shown in Table 11.

Seropositivity

In the present study seropositivity of dengue was 35.38%, which is in accordance with Ukey *et al.,* (31.3%), Sandhya Bhat *et al.,* (32.1%), Kalyanarooj *et al.,* (35%) Deora *et al.,* (40.97%), Tathe *et al.,* (39.14%) (Ukey *et al.,* (2010), Sandhya Bhat *et al.,* (2014), Kalyanarooj *et al.,* (1997), Deora *et al.,* (2015) and Tathe *et al.,* (2013). Increase in positivity in the present study may partially be attributed to the rapid unplanned urbanization with unchecked construction activities and poor sanitation facilities contributing to fertile breeding grounds for mosquitoes. It is also true that alertness among medical fraternity and availability of diagnostic tests in hospitals have contributed to the increased detection of cases (Mehta *et al.,* 2014).

Clinical features in dengue infection

The most frequent symptoms observed in dengue positive cases in present study were fever (100%) and headache (77.53%).

Other symptoms observed in the present study by decreasing order were bodyache (66.67%), nausea and vomiting (47.82), joint pain (45.65%), abdominal pain (38.4%), retro-orbital pain (38.4%), rash (21.73%).

Similar findings were observed by following previous studies shown in Table 12. In the present study hepatomegaly was the commonest sign observed in 21(15.21%) cases. Ascites was noted in 16 (11.59%) cases followed by pleural effusion in 14 (10.14%) and splenomegaly in 11(7.97%) cases respectively.

Similar findings observed by Deora *et al.,* (2015), Singh *et al.,* (2005), Bhaskar *et al.,* (2010) Kumar *et al.,* (2015), Shah *et al.,* (2006).

Mortality in our study was 2.89% which was similar to Kumar *et al.,* (2010), Singh *et al.,* (2005) and Duthade *et al.,* (2013) who found mortality in 2.4%, 2.7% and 3.7%, cases respectively.

Classification of dengue

In our study out of 138 dengue positive cases, large proportion of cases i.e. 84 (60.87%) belonged to the category of dengue without warning signs, followed by 39 cases (28.26%) dengue with warning signs and 15 cases (10.87%) had severe dengue as shown in Table 11. The present study results were comparable with Deora *et al.*, (2015) who observed 51.69% dengue without warning signs, 34.75% dengue with warning signs and 13.55% severe dengue.

While Jain *et al.*, (2013) observed 76% dengue without warning signs, 20% dengue with warning signs and 3 % severe dengue.

Table.1 Seropositivity of dengue virus infection

Total No. of clinically suspected dengue cases	Dengue positive	
	No. of cases	Percentage
390	138	35.38%

Table.2 Serologically positive cases in Group A, Group B and Total positive cases

Group A Positive (RICT &/or NS1 ELISA) (180 Clinically Suspected Samples)	Group B Positive (RICT &/or IgM ELISA) (210 Clinically Suspected Samples)	Total Positive Cases (Total No. of clinically Suspected Samples 390)
55	83	138 (35.38%)

Table.3 Showing various clinical signs in dengue positive cases

Signs	Dengue positive cases (n=138)	Percentage
Hepatomegaly	21	15.21%
Ascites	16	11.59%
Pleural effusion	14	10.14%
Splenomegaly	11	7.97%

Table.4 Distribution of serologically positive dengue cases into three categories as per WHO guidelines 2009(12)

(n=138)

Categories of Dengue	Total no. of cases	Percentage
Dengue without warning signs	84	60.87%
Dengue with warning signs	39	28.26%
Severe dengue	15	10.87%
Total	**138**	**100%**

Table.5 Serologically dengue positive cases diagnosed by RICT

(n=111)

Parameter	Positive Number	Percentage
NS1 antigen only	27	24.32%
IgM antibody only	48	43.24%
IgG antibody only	3	2.70%
NS1 antigen +IgM antibody	14	12.61%
NS1 antigen +IgG antibody	6	5.40%
IgM antibody + IgG antibody	10	9.00%
NS1 antigen + IgM antibody + IgG antibody	3	2.70%
Total	**111**	**100%**

Table.6 RICT positivity in primary and secondary dengue infections

(n=111)

RICT	Primary dengue infection (%)	Secondary dengue infection (%)
	Cases (%)	Cases (%)
Positive for only NS1	27 (24.32%)	-
Positive for only IgM	48 (43.24%)	-
Positive for only NS1 +IgM	14 (12.61%)	-
Positive for only IgG	-	3 (2.7%)
Positive for only NS1 +IgG	-	6 (5.4%)
Positive for only IgM + IgG	-	10(9.0%)
Positive for only NS1 + IgM + IgG	-	3 (2.7%)
Total	**89 (80.18%)**	**22 (19.82%)**

Table.7 RICT positive test results by day of fever

Day after onset of fever	Number of sera tested	Total RICT NS1 Ag positive	Total RICT IgM Ab positive
1	10	3	0
2	25	5	0
3	39	12	2
4	48	15	3
5	58	7	4
6	72	4	24
7	60	2	18
8	38	2	10
9	25	0	10
10	15	0	4
Total	**390**	**50**	**75**

Table.8 RICT Positivity in Group A (1-5 days) and Group B (6-10 days)

Group	RICT NS1 Ag positive	RICT IgM Ab Positive
Group A	42 (84%)	9 (12%)
Group B	8 (16%)	66 (88%)
Total	**50 (100%)**	**75 (100%)**

p value <0.0001 (Statistically significant)

Table.9 Percentage of Positive results by NS1 ELISA and RICT for NS1 Ag (During 1-5 days of Fever) in Group A

Test	Total NS1 ag positive	Percentage
RICT for NS1 Ag	42	23.34% (42/180)
NS1 ELISA	55	30.55% (55/180)

Table.10 Percentage of Positive results by IgM Capture ELISA and RICT for IgM Ab (During 6-10 days of fever) in Group B

Test	Total IgM positive	Percentage
RICT for IgM Ab	66	31.42% (66/210)
IgM capture ELISA	83	39.52 % (83/210)

Table.11 Showing NS1 ELISA Positivity for NS1 Ag and IgM Capture ELISA Positivity for IgM Ab

ELISA	Positive Results	Positivity
NS1 ELISA	55	30.55% (55/180)
IgM Capture ELISA	83	39.52% (83/210)

Table.12 Showing occurrence of common symptoms in various studies among dengue positive cases

Symptoms	(Chatterjee *et al.*, 2014)	(Kashinkunti *et al.*, 2013)	(Sreejith *et al.*, 2014)	(Kauser *et al.*, 2014)	(Dash *et al.*, 2005)	(Neeraja *et al.*, 2006)	(Kumar *et al.*, 2010)	(Deora *et al.*, 2015)	Present study
Fever	98%	100%	100%	100%	100%	100%	99.1%	100%	100%
Headache	90%	90%	92%	75.34%	86%	74%	47.6%	67.79%	77.53%
Bodyache	86%	81%	89%	32.87%	70%	53%	64.6%	54.23%	66.67%
Nausea/ vomiting	24%	56%	79%	57.53%	55%	62%	47.6%	48.3%	47.82%
Joint pain	-	-	86%	13.69%	55%	-	-	46.61%	46.65%
Abdominal pain	42%	48%	-	26.02%	37%	22%	37.6%	39.83%	38.40%
Retro-orbital pain	90%	-	44%	12.32%	-	7%	-	38.98%	38.40%
Rash	28%	20%	-	-	56%	41%	21.7%	23.72%	21.73%
Hemorrhagic /Bleeding manifesta-tions	23%	21%	6%	9.58%	-	7%	-	16.94%	15.21%

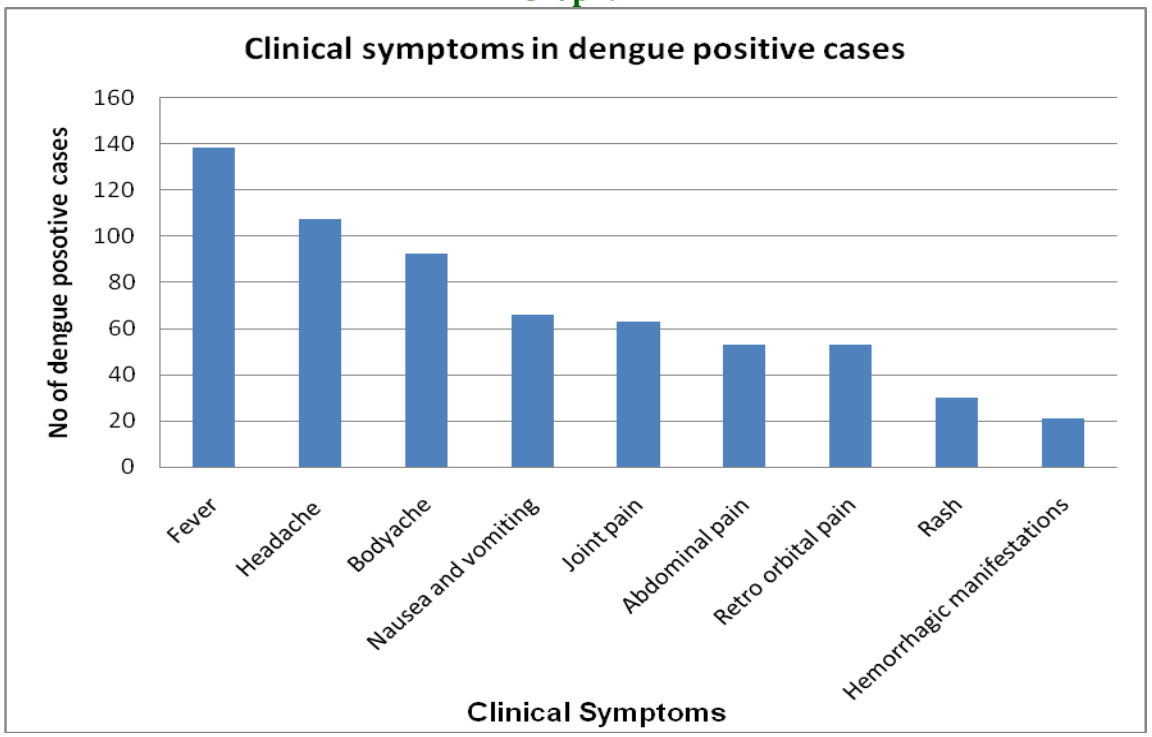
Table.13 Showing only NS1 positive cases diagnosed by RICT in various studies

Names of studies	(Ghosh *et al.*, 2013)	(Tabasum *et al.*, 2014)	(Shah *et al.*, 2015)	(Tathe *et al.*, 2013)	(Deora *et al.*, 2015)	(Kulkarni *et al.*, 2011)	(Golia *et al.*, 2012)	Present study
Only NS1ag Positive cases diagnosed by RICT (%)	51.56%	43.24%	46%	36.55%	29.09%	30%	23.3%	24.32%

Table.14 NS1 ELISA Positivity for NS1 Ag and IgM ELISA Positivity for IgM Ab in various studies

Studies	(Shrivastava *et al.*, 2011)	(Solanke *et al.*, 2015)	(Patil *et al.*, 2014)	(Ahmed *et al.*, 2015)	(Sahu *et al.*, 2015)	(Manohar *et al.*, 2015)	(Datta *et al.*, 2010)	(Kassim *et al.*, 2011)	(Solanke *et al.*, 2013)	Present study
NS1 ELISA positivity	26%	30.3%	_	_	36.02%	29.2%	23.3%	32.2%	28.4%	30.55%
IgM ELISA positivity	_	_	35.58%	38.9%	18.02%	48.9%	39.1%	40.9%	40.9%	39.52%

Graph.1



RICT results

Table 13 shows serologically positive cases diagnosed by RICT. Out of 111 cases positive by RICT, 27 cases (24.32%) were positive for NS1 antigen only.

Results of the present study were consistent with following studies shown in Table 13. NS1 Ag was also positive in combination with IgM Ab (12.61%), IgG Ab (5.4%) and IgM+IgG Ab (2.7%) by RICT as shown in Table 13. Thus in the present study NS1 antigen in combination with IgM Ab, IgG Ab and IgM Ab + IgG Ab was positive in 20.71% of the cases.

Similar results observed by Shah *et al.,* (2015) who found NS1 antigen in combination with IgM Ab, IgG Ab and IgM Ab+ IgG Ab was positive in 16% of the cases. In the present study IgM Ab positivity by RICT was 43.24%.

Results of the present study were consistent with that of Deora *et al.,* (2015), Kulkarni *et al.,* (2011) and Golia *et al.,* (2012) who found only IgM positivity by RICT in 43.63%, 50% and 33.3% of the cases respectively.

IgM Ab was also positive in combination with NS1 antigen (12.61%), IgG Ab (9.0%) and NS1+IgG Ab (2.7%) by RICT as shown in Table 13.

Thus in the present study IgM Ab in combination with NS1 antigen, IgG Ab and NS1 Antigen+IgG Ab was positive in 24.31% of the cases.

Similar results observed by Shah KD *et al.,* who found IgM Ab in combination with NS1 antigen, IgG Ab and NS1 Antigen + IgG Ab was positive in 26% of the cases (Shah *et al.,* 2015).

Primary and secondary infection

In the present study, 89 (80.18%) primary dengue infection and 22 (19.82%) secondary dengue infection cases were detected.

Similar findings were observed by Jayasimha *et al.,* (2012) who also found 82.6% primary dengue infection and (17.3%) secondary dengue infection cases.

Shah *et al.,* (2015) and Saini *et al.,* (2013) found 85%, 84.6% primary dengue infection and 15%, 15.3% secondary dengue infection cases respectively.

RICT NS1 Ag and IgM Ab positivity according to days of fever

Table 9 shows NS1 antigen was detectable in patient's sera from day one of fever, IgM antibodies from day 3 onwards and NS1 Ag was detected in patients sera till the 8th day of fever by RICT.

The present study findings were in tune with that of Deora *et al.,* (2015) and Chakravarti *et al.,* (2011) who detected NS1 Ag from day one of fever, IgM antibody from day three of symptoms and NS1 Ag detected upto the 8th and 7th day of fever respectively. Shah *et al.,* (2015) who detected NS1 Ag till 7th day of fever while Alcon *et al.,* (2002) and Kassim *et al.,* (2011) found NS1 Ag in patients sera till day 9thof fever. In our study NS1 Ag was positive for about 50 number of patients with a maximum positivity on 4th day (30%) followed by on 3rd day (24%). Similar findings observed by Patel *et al.,* (2013) who found maximum NS1 Ag positivity on day 4th day (61%) followed by 3rd day 16.7%. While Chithambaram *et al.,* (2014) found maximum NS1 Ag positivity on day 4-5 (61.2%) and Deora *et al.,* (2015) found maximum NS1 Ag positivity on 5th day (24.44) followed by 6th (20%), 4th day (13.34%).

IgM was positive for about 75 number of patients with a maximum positivity on 6th day (32%) and 7th day (24%). Similar findings observed by Deora *et al.,* (2015) and Kassim *et al.,* (2011) who found maximum IgM positivity on 5th and 6th day. While Shah KD *et al.,* found maximum IgM positivity on 5th day (Shah *et al.,* 2015)

RICT NS1 Ag and IgM positivity in group A and group B

In our study RICT NS1 ag positivity was 84% in Group A (1-5 days) and 16% in Group B (6-10 days) (Table 10). The NS1 Ag detection rate decreased from 84% in 1-5 days sera to 16 % in 6-10 days sera. Similar results observed by Datta *et al.,* (2010) who found 71.42% NS1 Ag positive were from acute phase serum samples and 28.4% were from early convalescent phase. Shah *et al.,* (2015) found NS1 ag was positive in 17.7% cases between 1-3 days, 61.2% cases between day 4-5, 20.9% cases positive between 6 - 7days.

Solanke *et al.,* (2015) found the NS1 Ag positivity also decreased from 47.3% on days 1-3 to 39.4% on days 4-6 and further decreased to 13.1% on days 7-9. Solanke *et al.,* (2013) found NS1 Ag positive in 70.73% cases on day 1 & 2 and decreased upto 4.87% on day 5.

In our study RICT IgM positivity was 12% in Group A (1-5 days) and 88% in Group B (6-10 days) (Table 10). The IgM Ab detection rate increased from 12% in 1-5 days sera to 88% in 6-10 days sera.

Similar results observed by Datta *et al.,* (2010) who found 93.61% IgM positive samples were from early convalescent phase and only 6.38% were positive from acute phase sera.

Solanke *et al.,* (2013) found IgM positive in 5.06% cases from day 5 onwards and gradually increased up till day 9 in 50.54% of cases.

NS1 ELISA positivity for NS1 Ag and IgM ELISA Positivity for IgM Ab

In the present study 55 Samples were positive by NS1 ELISA and NS1 ELISA positivity for NS1 Ag (For Group A) was 30.55% where as 83 samples were positive by IgM Capture ELISA and IgM ELISA positivity for IgM Ab was 39.52%which was similar to following studies shown in Table 14.

NS1 protein was found to be highly conserved for all dengue serotypes, circulating in high levels during the first few days of illness. The dengue NS1 antigen was not found in patients with Japanese encephalitis virus or yellow fever virus infections thereby implying that there is no cross-reaction of dengue NS1 protein with those of other related flaviviruses. Thus detection of NS1 has been a promising test to diagnose dengue in its early febrile stage (Shrivastava *et al.,* 2011).

Results of higher percentage of IgM positivity in the present study was because 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 (Patil, 2014).

RICT provide the useful aids in screening and ELISA should be considered as the confirmatory test in diagnosis of dengue.

NS1 ELISA has promising results in early diagnosis of dengue and when used in combination with IgM Capture ELISA it increases the diagnostic efficiency.

Early diagnosis of dengue helps to reduce the indiscriminate use of antimalarials and antimicrobials for treatment of patients with febrile illness.

Hence involvement of good laboratories for early and prompt diagnosis of dengue infection, coupled with vector control programmes and inducing awareness among the public, is needed in combating future epidemics of dengue.

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