CLINICOSEROLOGICAL DIAGNOSIS OF DENGUE FEVER

[1]Pooja Shah,[2] Nasira Sheikh, [3]Kishor Ingole

[1]Assistant Professor, [2]Professor and Head, [3]Professor and Head.

This study was conducted at GMC Solapur as a thesis topic of Dr. Pooja Shah under guidance of Dr. Nasira Sheikh and Dr. Kishor Ingole was HOD at that time.

Dr. Pooja G. Shah- Assistant Professor, Department of Microbiology, GMC Akola, Maharashtra, India.

ABSTRACT-

Introduction- Dengue is an endemic vector borne viral disease which is transmitted by bite if Aedes mosquito. Diagnosis of Dengue by viral isolation is cumbersome and is not practiced so, ELISA and rapid immunochromatography are mainstay of diagnosis.

Aims and objectives-

1. To assess the occurrence of seropositivity in clinically suspected Dengue patients at Tertiary care hospital.
2. To determine the occurrence of primary and secondary Dengue infection amongst clinically suspected cases of Dengue.
3. To evaluate utility of serodiagnosis and correlate results with clinical and laboratory profile.
4. To evaluate the efficacy of NS1 Antigen (Ag) assay as an early marker for Dengue virus infection.
5. To assess the seasonal pattern of Dengue over the study period at Tertiary care hospital.

Material and methods- Blood samples were taken from 350 clinically suspected Dengue cases. Panbio ELISA kits were used for IgM, IgG antibodies and NS1 antigen testing. J Mitra kits were used for Rapid immunochromatographic testing.

Results- 35.43% were serologically positive for Dengue. 16-30 years was most commonly affected age group. Males were more affected than females.Most of the cases were seen in November. Headache was most common symptom. Platelet count was <1,00,000/µL in 73.39% cases. Maximum numbers of cases were of Dengue with warning signs i.e. 54.03%.ELISA was more sensitive than Rapid test. Secondary Dengue was more common than primary Dengue. NS1 proved an important tool for diagnosis of early dengue infection.

Conclusion- Correlation of clinical symptoms is necessary along with laboratory tests.

Key words- Aedes, Dengue, ELISA, NS1, Platelet, Secondary Dengue

Manuscript-

Introduction- Dengue is most important arthropod borne viral disease in tropical and subtropical regions of world. Incidence of Dengue has increased dramatically in recent years. It has affected South East Asian region. Countries of South East Asian region are divided into 3 categories. India is included in Category A. Characteristics of Category A are[1]

a) Dengue is major public health problem.
b) Leading cause of hospitalization and death amongst children.
c) Hyperendemicity with all 4 serotypes circulating in urban areas.
d) It has also spread to rural areas.

Early diagnosis and proper treatment of Dengue is important as it may lead to complications like haemorrhage and shock if not treated and this can lead to high mortality and morbidity. Clinical profile does not always mimic diagnosis. So, confirmation by laboratory tests is very essential. There are laboratory tests which can detect Dengue fever in earlier stages of viremia and prompt treatment can be started in positive patients. Laboratory methods are broadly divided into-

Methods for Virus isolation-

1) Mosquito inoculation.
2) Animal inoculation
3) Cell culture.

Serological methods.-

1) Hemagglutination inhibition
2) Neutralization test
3) Complement fixation
4) ELISA
5) Rapid immunochromatography test (for which many commercial kits are available)
6) Polymerase chain reaction

In Maharashtra Dengue has been documented from Nagpur,[2] Parbani,[3] Dhule,[4] Pune.[5] Treatment of Dengue is largely symptomatic, and no vaccines have been licensed. Early detection and proper diagnosis of cases is of utmost importance. This study was undertaken to

a) To assess incidence of seropositivity in clinically suspected Dengue fever at tertiary care hospital.
b) To determine occurrence of primary and secondary dengue infection amongst clinically suspected cases of Dengue.
c) To evaluate utility of serodiagnosis and correlate results with clinical and laboratory profile.
d) To evaluate efficacy of NS1 Antigen (Ag) assay as an early marker for Dengue virus infection.
e) To assess seasonal pattern of Dengue.

Material and methods- Present study was conducted at a tertiary care teaching hospital in Department of Microbiology after receiving permission from institutional Ethical Committee. Study was carried out over a period from December 2013 to December 2015.

Inclusion criteria-

Patients admitted in Medicine and Pediatric department of a tertiary care hospital with clinical symptoms of Dengue as per 2009 WHO guidelines.

Exclusion criteria:

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

Type of study:

Cross sectional observational study.

Study population:

Total of 350 clinically suspected cases of both sexes with Dengue fever admitted in Medicine and Pediatric wards were included in study.

Clinical data:

After informed verbal consent, clinical history of suspected Dengue patients was taken from Medicine and Pediatric wards . Patients living in Dengue endemic area or travelled to Dengue endemic area, presenting with fever and any 2 of following findings- nausea, vomiting, rash, aches and pain, tourniquet test positive, leucopenia and any other warning sign were suspected as probable Dengue cases. 5-6 ml of venous blood was taken aseptically in plain bulb. Serum was separated from blood samples and transferred into labelled sterile bulbs.

All serum samples were tested by Rapid Immunochromatographic Card Test(ICT) (Jai Mitra and Co. Pvt. Ltd., New Delhi, India) and also by Dengue IgM antibody capture, Dengue IgG antibody capture ELISA and Dengue Early ELISA(Panbio).

Calculation of IgM:IgG ratio- Optical Density (OD) of IgM was divided by Optical Density (OD) of IgG and IgM:IgG ratio was calculated as per WHO guidelines 2009.

Blood sample for pathology:

Blood was sent in pathology department for doing Complete blood count. Platelet count, haematocrit values, and WBC count was calculated by cell counter method.

Statastical analysis-

Statistical analysis was done using Microsoft Excel and PASW (Predictive Analysis software) statistics version 18.

Results-

Table No.1:

Age and sex wise distribution in clinically suspected Dengue cases.

Age(Years)	Males n (%)	Females n (%)	Total N (%)
0-15	31(8.86)	17(4.86)	48(13.71)
16-30	53(15.14)	59(16.86)	112(32)
31-45	48(13.71)	41(11.71)	89(25.43)
46-60	27(7.71)	22(6.29)	49(14)
>61	34(9.72)	18(5.14)	52(14.86)
Total(n=350)	**193(55.14)**	**157(44.86)**	**350(100)**

Amongst 350, clinically suspected Dengue cases, 193(55.14%) were males and 157(44.86%) were females.

Table No. 2:

Serologically positive and negative Dengue cases amongst clinically suspected Dengue cases.

Clinically Dengue suspected cases	Total Dengue seropositive	Total Dengue seronegative
350	124(35.43%)	226(64.57%)

Table No. 3:

Age and sex wise distribution in Serologically Dengue Positive cases.

Age(Years)	Males n(%)	Females n(%)	Total n(%)
0-15	8(6.45%)	4(3.23%)	12(9.68%)
16-30	27(21.77%)	22(17.74%)	49(39.51%)
31-45	17(13.71%)	16(12.90%)	33(26.61%)
46-60	11(8.87%)	7(5.65%)	18(14.52%)
>61	9(7.26%)	3(2.42%)	12(9.68%)
Total (n=124)	**72(58.06%)**	**52(41.94%)**	**124(100%)**

In present study there were 39.51% serologically positive cases in age group of 16-30 years (n=49) followed by 26.61% in 31-45 years(n=33). Male:Female ratio was found to be 1.38:1.

Table No.4:

Showing month wise distribution pattern in serologically Dengue positive cases.

Month	Number of positive cases(n=124)	Percentage(%)
January	1	0.81
February	2	1.61
March	-	-
April	-	-
May	-	-
June	4	3.22
July	9	7.26
August	9	7.26
September	10	8.06
October	29	23.39
November	48	38.71
December	12	9.68

Chi Square (X^2) value 135.26 *df* 11 *P* : <0.001*.

Months of October and November had maximum number (62%) of positive cases as compared to other months. This was found to be statistically significant($p<0.001$).

Table No. 5:

Signs, symptoms and Pathological findings in Serologically Dengue positive cases

Signs,symptoms,pathological Finding	Serologically Dengue positive cases(n=124)	Percentage(%)
Fever	124	100
Nausea and Vomiting	56	45.16
Headache	89	71.77
Myalgia	61	49.19
Arthralgia	72	58.06
Rash	19	15.32
Retro-orbital pain	42	33.87
Abdominal pain	61	49.19

Haemorrhagic manifestations	20	16.13
Restlessness	12	9.68
Hepatomegaly	41	33.06
Spleenomegaly	7	5.65
Altered sensorium	9	7.26
Shock	12	9.68
Ascitis	14	11.29
Pleural effusion	7	5.65
Leucopenia	79	63.71

All serologically positive cases presented with fever i.e 100%. This was followed by headache in 71.77% cases.

Tables No. 6:

Platelet count in Serologically Dengue positive cases-

Platelet count	**Number (n=124)**	**Percentage %**
<1,00,000µl	91	73.39
>1,00,000 µl	33	26.61

Majority of Dengue positive cases (73.39%) had platelets <1,00,000. (26.61%) had platelet count >1,00,000.

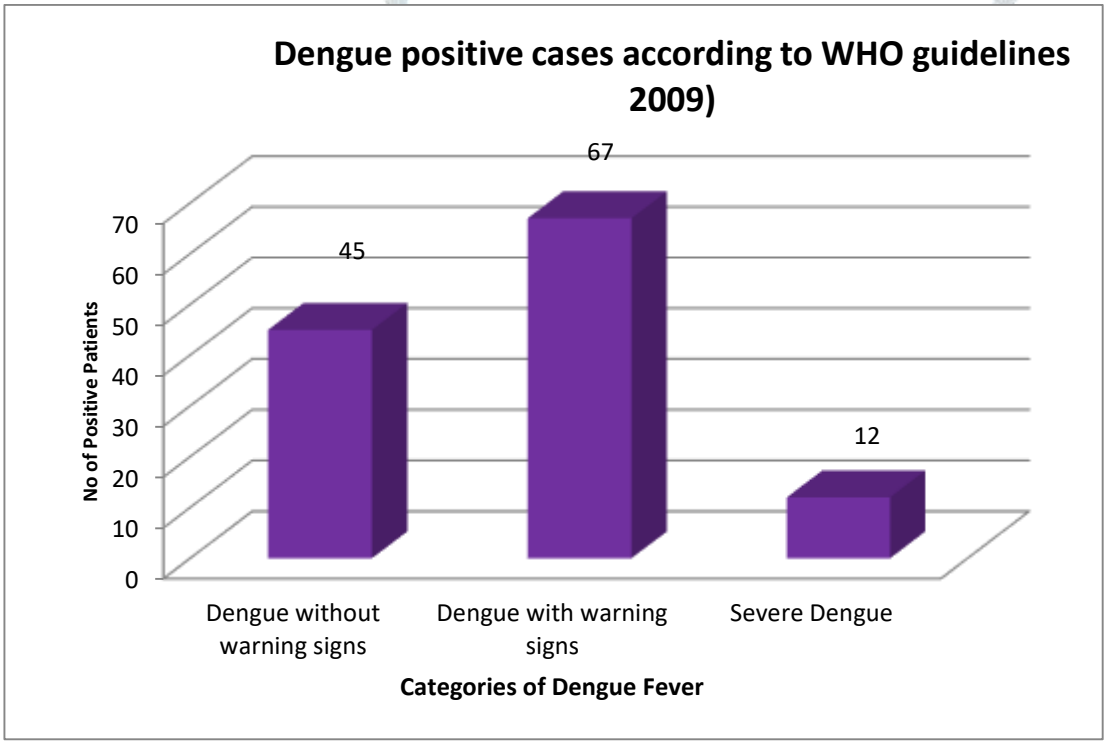

Figure 1: Division of serologically Dengue positive cases according to 2009 WHO guidelines.

Highest number of cases were with Dengue with warning signs i.e 54.03%.

Table No. 7:

Serologically Dengue positive cases by ELISA method –

Test type	**Results (n = 124)**	**Percentage (%)**
NS1 only	29	23.39
IgM only	26	20.97
IgG only	29	23.39
NS1+IgM	5	4.03
NS1+IgG	1	0.81

IgM+IgG	28	22.58
NS1+IgM+IgG	6	4.83

When ELISA was used, 23.39% samples were positive by NS1 alone and IgG alone. 20.97% were positive by IgM alone.

Table No. 8:

Serologically Dengue positive cases by Rapid immunochromatographic tests(ICT)-

Test type	**Resuls (n=103)**	**Percentage (%)**
NS1 only	26	25.24
IgM only	21	20.39
IgG only	25	24.27
NS1+IgM	3	2.91
NS1+IgG	1	0.97
IgM+IgG	24	23.31
NS1+IgM+IgG	3	2.91

When Rapid immunochromatographic tests were used, 25.24%samples were positive by NS1 alone, 20.39% by IgM alone, 24.27% by IgG alone.

Table No. 9:

Comparision of Rapid Immunochromatographic tests and ELISA amongst clinically suspected Dengue cases-

Method	**Rapid test positive n (%)**	**ELISA test positive n (%)**
NS1 only	26(7.43)	29(8.29)
IgM only	21(6)	26(7.43)
IgG only	25(7.14)	29(8.29)
NS1+IgM	3(0.86)	5(1.43)
NS1+IgG	1(0.28)	1(0.28)
IgM+IgG	24(6.86)	28(8)
NS1+IgM+IgG	3(0.86)	6(1.71)
Total	103/350(29.43%)	124/350(35.43%)

This table shows, 103 (29.43%) samples were positive by Rapid immunochromatographic tests and 35.43% samples were positive by ELISA.

Table No. 10:

Comparision of Rapid Immunochromatographic test with ELISA

Test	**Result by Rapid**	**Positive by ELISA**	**Negative by ELISA**	**Total**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**
Total NS1 Positive	Positive	33	0	33	80.49	100	100	97.48
	Negative	8	309	317				
	Total	**41**	**309**	**350**				
Total IgM positive	Positive	51	0	51	78.46	100	100	95.32
	Negative	14	285	299				
	Total	**65**	**285**	**350**				
Total IgG positive	Positive	53	0	53	82.81	100	100	96.30
	Negative	11	286	297				
	Total	**64**	**286**	**350**				

NPV= Negative Predictive Value, PPV= Positive Predictive Value.

All samples that were positive by Rapid tests were also positive by ELISA. Also rapid tests did not show any false positive cases(i.e. rapid positive , ELISA negative). Hence specificity and Positive predictive value(PPV) of all rapid tests was found to be 100%.

Table No. 11:

Number of primary and secondary Dengue cases according to 2009 WHO guidelines.

IgM/IgG OD ratio	Number(n=95)	Percentage
>1.2(Primary Dengue)	31	32.63%
<1.2(Secondary Dengue)	64	67.37%

Chi Square (X^2) value 11.463 *df* 1 *P* : 0.001*

Number of cases of secondary Dengue virus infection were 67.37% and Primary Dengue viral infection were 32.63%.This was found to be statistically significant(p<0.001).

Table No. 12:

Comparision of NS1 ELISA, IgM ELISA and IgG ELISA on basis of day of fever.

Day of fever	Total number of samples collected	Samples positive by NS1 ELISA	Samples positive by IgM ELISA	Samples positive by IgG ELISA
1	11	3	-	-
2	26	5	-	-
3	39	8	11	11
4	40	10	13	14
5	52	7	17	18
6	47	4	9	7
7	43	2	5	7
8	41	1	4	3
9	37	1	3	2
10	14	-	3	2
Total	**350**	**41**	**65**	**64**

Chi Square (X^2) value 31.35 *df 18 P* : 0.026*.

This differential positivity of tests on different days of fever was found to be statistically significant(p<0.001).

Discussion- In present study males (55.14%) were predominant amongst clinically suspected as compared to females (44.86%) and 16-30 years age group was more clinically suspected (table1). In present study, number of clinically suspected cases in age group of 16-30 years was 32%. In study of Gunasekaran et al (2011)[6] males were more clinically suspected as compared to females like that of our study. There were 60.77% clinically suspected males and 39.23% clinically suspected females in that study. In study of Padhi et al (2014) [7] more clinically suspected cases were in 11-30 years age group. In that study 49% cases were suspected in age group of 11-30 years.

In present study, seroprevalence of Dengue in our area was found to be 35.43% (table2). Our findings regarding seroprevalence are similar to following studies-

Name of study	Seroprevalence
Present study	35.43%
Kalyanarooj et al (1997)[8]	34.88%
Lakshmi P.et al (2014)[9]	34.17%
Ukey et al(2010)[2]	31.3%
Saini et al(2013)[10]	30.64%.

Seroprevalence in other studies are- Kulkarni et al(2011)[11] 15.21%, Garg A et al(2011)[12] 19.72%, Patankar et al(2014)[13] it is 21.06%, Gupta et al(2006)[14] 44.56%.

Increase in seroprevalence may be due to poor sanitation, which favours growth of mosquito, rapid unplanned urbanization with unchecked construction activities and increase in detection rate. Decrease in seroprevalence may be due to increase awareness amongst people through mass media, early diagnostic facilities which helps in early detection of cases.

In present study, table 3, highest number of Dengue positive cases 39.51% (n=49) were found in age group of 16-30 years which correlates with study of Kaup S.et al (2014) [15] in which 54.94% cases were found in age group of 16-30 years, Ukey et al (2010) [2] in which 31.71% cases were in age group of 15-30 years. Similar findings are as follows-

Study	**Finding**
Present study	16-30 years (39.51%)
Kaup S.et al(2014)[15]	16-30 years (54.94%)
Ukey et al(2010)[2]	15-30 years (31.71%)
Patankar et al(2014)[13]	18-35 years(58%)
Saini et al (2013)[10]	15-44 years (50.89%)
Stephen S et al (2014)[16]	21-40 years (48.6%)
Bansal et al (2014)[17]	17-44 years(66.04%)
Pardeshi et al (2014)[18]	21-30 years(28.81%)

More incidence in Pediatric age group was found in study of Sarkar et al (2012), [19] Dash et al(2005)[20] and Pandey et al(2012).[21]

In present study there were more cases in 16-30 years followed by 31-45 years. This is mainly due to fact that this age group is mostly working population and is more exposed to outside environment.

In our study, male: female ratio was 1.38:1.

Study	**Result of M:F ratio**
Present study	1.38:1
Pardeshi et al(2014)[18]	1.28:1
Kumar et al(2010)[22]	1.82:1
Bansal et al (2014)[17]	1.98:1
Lakshmi et al (2006)[23]	2:1
Sood et al (2013)[24]	2.43:1

Higher incidence amongst females were noted in study of Fazal et al (2015)[25], and Murugananthan et al(2014)[26]. Reason of increase in incidence in males could be more exposure to outside environment, clothing style. And females are less reported because lower reporting and less care seeking behaviour of women.

In present study, in months of October and November we found maximum number (62%) of positive cases as compared to other months(Table 4). This was found to be statistically significant ($p<0.001$). In a study of Amin et al (1999),[27] most number of cases(72.77%) were found from October to December i.e postmonsoon period. Other studies in which same findings were found are- Patankar et al(2014)[13] in which most of cases were found in October followed by September and November, Ukey et al(2010)[2] in which most of cases were found in September to November. Gupta et al (2006)[14] most cases were found from September to November with peak in October.

In present study maximum number of cases (38.71%) were found in month of November followed by October (23.39%).An increase in number of cases were noted from month of July. This may be due to fact that, rainy season favours formation of stagnant water. And this is followed by breeding of *Aedes* mosquito at that site. According to Jayasimha et al, breeding of *Aedes aegypti* is highest during pre and post monsoon period.[28] But sporadic cases extend up to December which indicates endemicity of infection up to December.[28]

In present study, table 5,shows, signs, symptoms and pathological findings in Dengue positive cases. In present study, fever was found in 100% cases, nausea and vomiting in 45.16% cases, myalgia in 49.19%, headache in 71.77%, arthralgia in 58.06%, rash in 15.32%, retro-orbital pain in 33.87%.

In our study fever was seen in 100% cases which correlates with study of Bansal et al,[17] Dash et al,[20] Singh et al[29], Kale et al[30]. In all these studies, fever was found in 100% cases.

In present study, headache was next predominant symptom which was found in 71.77% cases which is like that of study of Deora et al[31] and Lakshmi V et al [23] in which it was found in 67.79% and 74% cases respectively. In present study, Nausea and Vomiting was reported in 45.16% cases which is comparable to studies of Kumar MP et al[32], Kumar A et al[22], Richards et al[33] and Deora et al[31] in which it was 47.1%, 47.6%, 47.8% and 48.30% respectively. In present study, myalgia was observed in 49.19% cases which is similar to studies of Deora et al[31] and Sharma et al[34] in which it was 54.23% and 45.9% respectively.

In present study, arthralgia was found in 58.06% cases which is similar to studies of Dash et al (55%)[20] and Fazal et al (54%)[25]. In present study, rash was found in 15.32% cases. In a study of Kumar A et al rash was reported in 21.7%. In present study, retro-orbital pain was seen in 33.87% cases which is comparable to study of Deora et al[31] in which it was found in 38.98% cases.

In present study, abdominal pain was most common warning sign which was in (49.19%) cases which is comparable to study of Fazal et al[25], and Aggarwal et al[35] in which it was 48% and 49% respectively. In present study, haemorrhagic manifestations were seen in 16.13% cases which are like that of study of Deora et al[31] and Kumar MP[32] et al in which it was 16.94% and 19.5%. In present study, hepatomegaly was found in 33.06% cases. In study of Kumar MP[32] et al it was found in 27.1% cases. In our study spleenomegaly was found in 5.65% cases. This correlates with study of Bansal et al[17] in which it was found in 9.8% and Sharma et al[34] in which it was 8.2%.

In present study, ascitis was found in 11.29% cases which match with study of Jain et al[36] in which it was found in 10% cases. Pleural effusion in present study was reported in 5.65% cases which is somewhat similar to study of Singh et al [29] in which it was 1.08% and Sharma et al [34] in which it was 8.2%. In present study, altered sensorium and restlessness was noted in 7.26% and 9.68% respectively. In study of Kumar A et al[22] and Aggarwal et al[35], altered sensorium was found in 10.3% and 4% respectively. In study of Deora et al[31], restlessness was found in 7.62%.

Shock was seen in 9.68% cases in present study which co-incides with study of Kale et al[30] (10%).In study of Fazal et al[25], Shock was found in 8% cases. Lower incidence of shock was found in study of Deora et al[31] and Bansal et al[17]. This could be due to fact that, in study of Deora et al[31] there were more number of primary dengue cases as compared to secondary dengue cases. In our study, leucopenia was seen in 63.71% cases. In a study of Jain et al[36] and Carrasco et al[37] , leucopenia was seen in 62.07% and 79.36% cases respectively.

Study	**Platelet count <1,00,000(%)**
Present study	73.39%
Fazal et al(2015)[25]	73%
Lakshmi P et al(2014)[9]	78%
Tathe et al(2013)[38]	81.72%
Bansal et al(2014)[17]	80.11%
Kulkarni et al (2011)[11]	68.75%.

In our study 2009 WHO classification was used because according to WHO guidelines of year 2009,[39] there were many reports of difficulties in use of 1997 classification, difficulties in applying criteria for DHF in clinical situation, together with increase in clinically severe Dengue cases which did not fulfil strict criteria of DHF, led to request for classification to be reconsidered.[39]

According to, Macedo et al (2014)[40], has demonstrated superiority of revised classification (WHO 2009) for detection of severe cases among hospitalized children with laboratory-confirmed Dengue. According to them, revised scheme had a significantly better sensitivity (86.8%; P value <0.001) than traditional scheme WHO 1997 scheme (62.3%). Similar findings were seen in study of Narvaez et al (2011)[41]

In our study, highest number of cases were from Dengue with warning signs n=67 i.e 54.03% (figure 1), this was followed by Dengue without warning signs and that was in 36.29% and severe Dengue was found in 9.68% cases. In study of Kale et al(2014)[30], maximum number of cases were of Dengue with warning signs i.e 85.33%, shock was seen in 10% cases. These findings correlate with our study. In study of Deora et al[31] maximum number of cases (51.69%), were of Dengue without warning signs, whereas shock was found in 13.55% cases and Dengue with warning signs was in 34.75% cases. In study of Arunagirinathan et al,[42] children with Dengue without warning signs were (38%), Dengue with warning signs were(52%), and severe Dengue were (10%) of cases.

In our study more number of severe Dengue cases could be due to, more number of secondary Dengue infections as compared to primary Dengue infection.

In present study (table 7), NS1 alone was positive only in 23.39% by ELISA which correlates with study of Wattal et al(2010)[43] in which he divided his study in 3 groups. In group 1, NS1by ELISA was positive in 23.3% cases. Our study is also similar to Shrivastava et al(2011)[44] in which it was positive in 26% cases by Panbio NS1 Capture ELISA. In study of Kassim et al(2011)[45],it was positive in 32.2% cases by NS1 ELISA . In present study, IgM alone by ELISA was positive in 20.97% cases which correlates with study of Padhi et al (2014)[7] in which it was found to be 21.05%. In a study of Kale et al(2014)[30] it was found in 28.67% cases. In our study, by IgG ELISA alone was detected in 23.39% patients and NS1+ IgM was detected in 4.03% patients which correlates with study of Lakshmi P. et al(2014)[9] in which they were found in 18.75% and 6.25% respectively . In a study of Hati et al (2014),[46] in panel 3, IgG was found in 34.2% cases and NS1+IgM was found in 8.8% cases by ELISA. In our study IgM+IgG by ELISA was found in 22.58% cases. In study of Golia et al (2012)[47] IgM+IgG positive samples by ELISA were 14.7%. In our study, only 1 sample was positive for NS1+IgG (0.81%) by ELISA. In study of Lakshmi P et al (2014)[9] NS1+IgG by ELISA was found in 3.13% cases. Triple positive i.e NS1+IgM+IgG by ELISA in our study was found in 6 samples i.e 4.83%. This correlates with study of Hati et al(2014)[46] in which in panel 3 NS1+IgM+IgG was found in 5.1% cases.

In our study (table 8) shows NS1 alone by Rapid Immunochromatographic tests was positive in 25.24% cases which correlates with study of Lakshmi P et al(2014) , Golia et al(2012) and Deora et al(2015) in which it was 24%, 23.3%, and 29.09% respectively.[9,47,31] In present study, IgM alone by Rapid ICT was positive in 20.39% cases which was similar to study of Tathe et al(2013)[38] in which it was 17.20%. In our study, IgG alone by Rapid ICT was positive in 24.27% cases which was similar to studies of Lakshmi P et al(2014), and Tathe et al(2013) in which it was 24% and 21.50 respectively.[9,38,] In our study, NS1+IgM by rapid ICT were found in 2.91% cases which correlate with study of Khan A (2014) et al and Saini et al(2013)in which it was 3.7% and 4.27% cases respectively.[48,10] In present study, NS1+IgG by rapid ICT was found in 0.97% cases which are like that of studies of Saini et al(1.42%), Golia et al(1.1%) cases.[10,47] In present study, NS1+IgM+IgG by rapid ICT was found in 2.91% cases. In study of Deora et al(2015)[31] NS1+IgM+IgG was present in 1.81% cases.

In present study, table 9 shows that, number of samples positive by ELISA were more as compared to rapid tests. Out of 350 clinically suspected Dengue cases, 35.43% cases were positive by NS1 and/or IgM and/or IgG ELISA and 29.43% cases were positive by Rapid tests. And in our study, all markers which were positive by Rapid ICT were also positive for ELISA i.e not a single case detected by Rapid test was missed by ELISA. This is also seen in study of Lakshmi P et al(2014) [9] except for NS1 ELISA. In study of Lakshmi P et al(2014) [9] 48% of samples were positive by ELISA and 31% by Rapid tests. In her study, NS1 by Rapid ICT were more as compared to ELISA.

In a study of Shrivastava et al(2011)[44], 91 samples were tested for NS1 ELISA and NS1 Rapid test. In his study 26% of samples were positive by ELISA and only 16.5% of samples were positive by Rapid ICT. All samples that were positive by ELISA were also positive by Rapid tests. In our study only 1 sample was positive for NS1+IgG by ELISA and also positive by Rapid ICT. This correlates with study of Chakraverti et al [49] in which all samples positive for NS1+IgG ELISA were also positive for NS1+IgG Rapid ICT.

In present study, table 10, sensitivity, specificity, Positive Predictive Value and Negative Predictive value of rapid test were calculated taking ELISA as standard because according to literatures, ELISA is more sensitive as compared to Rapid Immunochromatographic tests.[49] In present study, rapid tests did not show any false positive cases (i.e. rapid positive, ELISA negative). Hence in our study, specificity and Positive predictive value (PPV) of all rapid tests was found to be 100%. Similar results with 100% specificity and Positive Predictive Value were found in study of, Lakshmi P et al.[9]

In present study, sensitivity of NS1, IgM and IgG by Rapid Immuno-chromatographic tests was 80.49%, 78.46% and 82.81% respectively as compared to ELISA. Negative Predictive Value (NPV) was 97.48%, 95.32% and 96.30% for NS1, IgM and IgG Rapid immunochromatographic tests respectively. In study of Lakshmi P et al[9], sensitivity for NS1 rapid , IgM rapid and IgG rapid was 81.8%, 42.9% and 52% respectively and Negative predictive values for NS1 rapid, IgM rapid and IgG rapid was 96%, 76.5% and 75.8% respectively. Difference in sensitivity and specificity in various studies may be due to different companies of kits used. It may be also due to cross reactivity amongst Flaviviruses in Dengue endemic areas.[50]

According to Chakraverti T et al[49], Golia et al (2012)[47] ELISA was more sensitive than rapid tests. In a study of Jayasimha et al[28] taking ELISA as gold standard, Sensitivity of rapid was 80.66%,Specificity was 100%, Positive Predictive Value was 100% and Negative Predictive Value was 72.4% . In study of Shrivastava et al[44] who compared Rapid NS1 test taking ELISA as gold standard results of their study were, Sensitivity 62.5%(15/24),Specificity100%(67/67), PPV 100% (15/15) , NPV 88.15 % (67/76). Results are like that of our study in which specificity and PPV are 100% and sensitivity of ELISA is more than Rapid ICT tests.

In present study, all samples that were positive by IgM or/and IgG ELISA were subjected to IgM/IgG ratio to determine if they belong to primary Dengue infection or secondary Dengue infection. Innis et al used IgM and IgG capture ELISA tests to detect both Dengue IgM and IgG antibodies.[51] Kuno et al used IgM capture method and classical indirect ELISA method, different approach from Innis, for measuring Dengue IgM and IgG, respectively, to define primary versus secondary infections.[51] Commercial capture ELISA for detecting Dengue-specific IgM and IgG antibodies (PanBio Dengue Duo, Australia) showed excellent sensitivity and specificity for distinguishing primary and secondary infections.[51] According to Perng et al(2013), [52] besides use of IgM/IgG ratio, it is difficult at best to distinguish between primary and secondary infection.[52]

Primary and secondary infection were distinguished on basis of OD(optical Density) ratio of IgM/IgG and if OD value ratio was >1.2 it was primary Dengue virus infection and if ratio of IgM/IgG was <1.2 it was secondary Dengue virus infection according to WHO guidelines 2009.[53] Pe Yun Shu et al has suggested this is best cut off ratio to distinguish primary and secondary Dengue viral infection. [54]

In study of Atul Garg et al(2011)[12], using Panbio Dengue IgM and IgG Capture ELISA kits when same ratio of 1.2 was used, number of primary Dengue cases were 8% and secondary Dengue cases were 92%.This correlates with our study in which secondary Dengue cases were more.

Similar to our study, Pei Yun Shu et al(2003)[54] used same IgM:IgG ratio of 1.2 to distinguish primary and secondary Dengue virus infection . If OD (optical Density) ratio of IgM/IgG was >1.2 it was primary Dengue virus infection and if ratio of IgM/IgG was <1.2 it was secondary Dengue virus infection. In that study, cases with primary Dengue infection were more as compared to secondary Dengue virus infection.

In study of Chan K-S et al (2009)[55] RT PCR was done for Dengue virus serotyping and IgM:IgG ratio was used to differentiate primary and secondary Dengue virus infection. Infection by DENV-2 more commonly occurred as a secondary infection, while infection by DENV-3 was more common as a

primary infection ($p < 0.001$).In a study of Lin C et al (2010)[56] from 2002 to 2007, secondary infection was common in 2002 and in rest all years primary infection was more common.

In study of, Hung N et al (2004)[57], when same ratio was used on sera positive for IgM and/or IgG ELISA, primary infection was more common as compared to secondary dengue viral infection.

In present study, NS1 appears in serum from first day of infection and it is present till 9th day of infection. In study of Deora et al,[31] NS1 was present on day 1 and day 2 of fever and IgM and IgG antibodies were not detected at this time. This difference was statistically significant like that of our study. In that study also, IgM started appearing on 3rd day of fever like our study. Our results also correlates with study of Alcon et al[58] and Kassim et al[45] in which NS1 appears on 1st day. In study of Alcon et al,[58] NS1 was present till 9th day of fever whereas Kassim et al[45] found it was present till 8th day of fever. In our study, NS1 levels peaked at 3-6th day of fever. This correlates with study of Mahapatra et al[59] and Kassim et al[45] in which NS1 levels peaked at 3rd-5th day of fever. In our study, in first four days of fever NS1 was positive in 63.41% cases and after 4 days it was found in 36.59% cases. In study of Wattal et al[43], NS1 Ag positivity varied from 71.42% to 28.4% in acute and early convalescent sera. In that study acute phase sera were collected 1-4 days after onset of illness and early convalescent sera were collected > 5 days of illness.

In present study, IgM and IgG antibodies appears in serum from third day of infection and it is present in our study till 10th day of infection. In study of Mishra et al,[60] most of samples were IgM and IgG antibodies positive from day 3 of illness. Majority of samples that were positive for IgG were from day 3 of fever and this was statistically significant like that of our study. In study of Palanivel et al[61] IgM started appearing from 4th day of fever. In study of Naz et al[62], IgG was detected in highest number on day 4-6 of fever. This result correlates with our study.

In our study NS1 only was positive in 23.39% cases. And NS1 with IgM and IgG was positive in 9.67% cases. These cases could have missed if NS1 was not studied.

Conclusion- Correlation of clinical symptoms with laboratory diagnosis is very important. 35.43% were serologically positive for Dengue. 16-30 years was most commonly affected age group. Males were more affected than females.Mostly cases were seen in November. Headache was most common symptom. Platelet count was <1,00,000/µL in 73.39% cases. Maximum numbers of cases were of Dengue with warning signs i.e. 54.03%.ELISA was more sensitive than Rapid test. Secondary Dengue was more common than primary Dengue. NS1 proved an important tool for diagnosis of early dengue infection.

Acknowledgment- We are thankful to Mr. Vilas Bhujal and Mr. Pathak for their constant technical support.

Conflict of interest- None

References-

1) Park K. Park's Textbook of preventive and social medicine, 23rd ed. Jabalpur, India: M/s Banarasidas Bhanot publishers; 2015 February. p769-772.
2) Ukey P, Bondade S, Paunipagar P, Powar R, Akulwar S. Study of seroprevalence of dengue fever in central India. Indian J Community Med. 2010 Oct-Dec; 35(4):517-519.
3) Mehendale SM, Risbud AR, Rao JA, Banerjee K. Outbreak of Dengue fever in rural areas of Parbhani district of Maharashtra, India. Indian J Med Res. 1991 Jan; 93:6–11.
4) Padbidri VS, Mahadev PV, Thakre JP, Pant U, Illkal MA, Varghese GG, et al. Virological and entomological investigations of an outbreak of Dengue fever in Dhule district, Maharashtra. Indian J Med Microbiol. 1996; 14(1):25–32.
5) Cecilia D, Kakade M, Bhagat A, Vallentyne J, Singh A, Patil J et al. Detection of dengue-4 virus in Pune, western india after an absence of 30 years - its association with two severe cases. Virology Journal. 2011; 8(1):46.
6) Gunasekaran P, Kaveri K, Mohana S, Arunagiri K, Suresh Babu BV, Padma Priya P et al. Dengue disease status in Chennai (2006-2008): A retrospective analysis. Indian J Med Res.2011 Mar; 133(3): 322-325.
7) Padhi S, Dash M, Panda P, Parida B, Mohanty I, Sahu S et al. A three year retrospective study on the increasing trend in seroprevalence of dengue infection from southern odisha, India. Indian J Med Res. 2014 Nov; 140: 660-664.

8) Kalayanarooj S, Vaughn D, Nimmannitya S, Green S, Suntayakorn S, Kunentrasai N et al. Early Clinical and Laboratory Indicators of Acute Dengue Illness. JID.1997 Aug; 176(2):313-321.
9) Lakshmi P, Nainar P. Challenges in the early diagnosis of dengue: A practical approach. J Sci Soc. 2014; 41(2):85-88.
10) Saini S, Kinikar A, Deorukhkar S, Bhalerao D, Roushani S. Epidemiology and seropositivity of dengue fever cases in a rural tertiary care hospital of western Maharashtra, India. Int J of Biomed & Adv Res. 2013; 4(7):473-477.
11) Kulkarni R D, Patil S S, Ajantha G S, Upadhya A K, Kalabhavi A S, Shubhada R et al. Association of platelet count and serological markers of dengue infection- importance of NS1 antigen. Indian J Med Microbiol 2011; 29(4):359-62.
12) Garg A, Garg J, Rao YK, Upadhyay GC, Sakhuja S. Prevalence of dengue among clinically suspected febrile episodes at a teaching hospital in North India. J. Infect. Dis. Immun.2011 May;3(5):85-89.
13) Patankar MC, Patel BV, Gandhi VP, Shah PD, Vegad MM. Seroprevalence of dengue in Gujarat, Western India: A study at a tertiary care hospital. Int J Med Sci Public Health. 2014; 3(1):16-18.
14) Gupta E, Dar L, Kapoor G, Broor S. The changing epidemiology of Dengue in Delhi, India. Virol J. 2006 Nov; 3: 92.
15) Kaup S, Sankarankutty J. Seroprevalence and Seasonal Trend of Dengue Virus Infection at a Teaching Hospital in Tumkur, India. Sch. J. App. Med. Sci. 2014; 2(3A):922-926.
16) Stephen S, Charles M, Anitharaj V, Deepa C, Umadevi S. Early dengue diagnosis by Nonstructural protein 1 antigen detection: Rapid immunochromotography versus two the enzyme-linked immunosorbent assay kits. Indian J Pathol and Microbiol. 2014; 57(1):81-84.
17) Bansal N, Uniyal N, Khrime D, Verma A. Clinical Profile and outcome of Dengue fever in Tertiary level Hospital in Uttrakhand, India. Transworld Medical Journal. 2014; 2(3):165-169.
18) Pardeshi A, Shinde R, Jagtap A, Kembhavi R, Giri M, Kavathekar S. Retrospective Cross-sectional Study of Dengue Cases in IPD with Reference to Treatment- Monitoring & Outcome in KEM Hospital, Mumbai. AJEID. 2014; 2(4):97-100.
19) Sarkar A, Taraphdar D, Chatterjee S. Molecular Typing of Dengue Virus Circulating in Kolkata, India in 2010. Journal of Tropical Medicine. 2012; 2012:1-5.
20) Dash PK, Saxena P, Abhayankar A, Bhargava R and Jana AM. Emergence of dengue virus type-3 in northern India. Southeast Asian J Trop Med Public Health 2005 Mar; 36(2):370-77.
21) Pandey N, Nagar R, Gupta S, Omprakash, Khan D, SinghDD et al. Trend of dengue virus infection at Lucknow, north India (2008- 2010): a hospital based study. Indian J Med Res. 2012; 136(5):862-867.
22) Kumar A, Rao C, Pandit V, Shetty S, Bammigatti C, Samarasinghe C. Clinical manifestations and trend of dengue cases admitted in a tertiary care hospital, Udupi district, Karnataka. Indian J Community Med. 2010 Jul; 35(3):386-390.
23) Lakshmi V, Teja V, Umabala P, Subbalakshmi M, Neeraja M. Serodiagnosis of dengue virus infection in patients presenting to a tertiary care hospital. Indian J Med Microbiol. 2006 Oct; 24(4):280-282.
24) Sood S. A Hospital Based Serosurveillance Study of Dengue Infection in Jaipur (Rajasthan), India. J Clin Diagn Res. 2013 Sep; 7(9):1917-1920.
25) Fazal F, Biradar S. Clinical and Laboratory Profile of Dengue Fever. J of Evidence Based Med & Healthcare. 2015; 2(9):1136-1147.
26) Murugananthan K, Kandasamy M, Rajeshkannan N, Noorden F. Demographic and clinical features of suspected dengue and dengue haemorrhagic fever in the Northern Province in Sri Lanka, a region afflicted by an internal conflict for more than 30 years- a retrospective analysis. International journal of infectious diseases(IJID).2014; 27:32-38.
27) Amin MMM, Hussain AMZ, Murshed M, Chowdhury IA, Mannan S, Chowdhuri SA et al. Sero-Diagnosis of Dengue Infections by Haemagglutination Inhibition Test (HI) in Suspected Cases in Chittagong, Bangladesh. Dengue Bulletin 1999; 23: 34-38.
28) Jayasimha VL, Thippeswamy MTR , Yogesh Babu KV , Vinodkumar CS , Niranjan HP , Raghukumar KG et al. DENGUE: SEROPREVALENCE, COMPARISON OF RAPID TEST WITH ELISA. National journal of basic medical sciences. 3(1):57-60.
29) Singh NP, Jhamb R, Agarwal SK, Gaiha M, Dewan R, Daga MK et al. The 2003 outbreak of Dengue fever in Delhi, India. Southeast Asian J Trop Med Public Health. 2005 Sep; 36 (5):1174-78.
30) Kale AV, Haseeb M, Sandeep Reddy C, Khan S, Golwalkar A, Badaam KM. Clinical Profile and Outcome of Dengue Fever from a Tertiary Care Centre at Aurangabad Maharashtra India: An Observational Study. IOSR-JDMS.2014 Sep; 13(9):14-19.

31) Deora(Kaswa) P, Bhalchandra M, Mulay V. Early diagnosis of Dengue- Need of the hour. National Journal of Medical Sciences.2015 Jan; 4(1):60-65.
32) Kumar MP, Kulkarni S, Rohit A, Bhandarkar A. Dengue- A clinic-epidemiological study in a tertiary care hospital of North Karnataka India. International Journal of Current Research. 2015 May; 7(5):16123-16126.
33) Richards AL, Bagus R, Baso SM, Follows GA, Tan R, Graham RR et al.The first reported outbreak of Dengue Haemorrhagic Fever in Irian Jaya, Indonesia. Am J Trop Med Hyg.1997 Jul; 57(1):49-55.
34) Sharma S, Sharma SK, Mohan A, Wadhwa J, Dar L, Thulkar S et al. Clinical profile of Dengue Haemorrhagic Fever in Adults during 1996-Outbreak in Delhi, India. Dengue Bulletin. 1998; 22:20-30.

35) Aggarwal A, Chandra J, Aneja S, Patwari AK, and Dutta AK. An epidemic of dengue haemorrhagic fever and dengue shock syndrome in children in Delhi. Indian Pediatr.1998 Aug; 35(8):727-32.

36) Jain A, Shah AN, Patel P, Desai M, Somani S, Parikh P et al. A clinico-hematological profile of Dengue outbreak among health-care professionals in a tertiary care hospital of Ahmedabad with analysis on economic impact. National J Community Med. 2013 Apr-Jun; 4(2):286-290.
37) Carrasco LR, Leo YS, Cook AR, Lee VJ, Thein TL, Go CJ et al. Predictive Tools for Severe Dengue Conforming to World Health Organization 2009 Criteria. PLoS Negl Trop Dis.2014; 8(7): e2972.
38) Tathe SS, Chincholkar VV, Kulkarni DM, Nilekar SL , Ovhal RS, Halgarkar et al. A study of NS1 antigen and platelet count for early diagnosis of dengue infection. Int.J.Curr.Microbiol.App.Sci. 2013; 2(12): 40-44.
39) World Health Organization(WHO). Regional office for Southeast Asia. Comprehensive Guidelines for Prevention and Control of Dengue and Dengue Haemorrhagic Fever, Revised and Expanded Edition. New Delhi. WHO; 2011. p1-212.
40) Macedo G, Gonin M, Pone S, Cruz O, Nobre F, Brasil P. Sensitivity and Specificity of the World Health Organization Dengue Classification Schemes for Severe Dengue Assessment in Children in Rio de Janeiro. PLoS ONE. 2014 Apr; 9(4):e96314.
41) Narvaez F, Gutierrez G, Pérez M, Elizondo D, Nuñez A, Balmaseda A et al. Evaluation of the Traditional and Revised WHO Classifications of Dengue Disease Severity. PLoS Negl Trop Dis. 2011 Nov; 5(11):e1397.
42) Arunagirinathan A, Thirunavukarasu B, Narayanaswamy DK, Raghavan A, Raghavendhran VD. Clinical Profile and Outcome of Dengue Fever Cases in Children by Adopting Revised Who Guidelines: A Hospital Based Study. Int J Sci Stud. 2015 May; 3(2):174-178.
43) Wattal C, Datta S. Dengue NS1 antigen detection: A useful tool in early diagnosis of dengue virus infection. Indian J Med Microbiol.2010 Apr-Jun; 28(2):107-110.
44) Shrivastava A, Dash P, Tripathi N, Sahni A, Gopalan N, Lakshmana Rao P. Evaluation of a commercial Dengue NS1 enzyme-linked immunosorbent assay for early diagnosis of dengue infection. Indian J Med Microbiol. 2011 Jan-Mar; 29(1):51-55.
45) Kassim FM, Izati MN, TgRogayah T, Apandi YM, Saat Z. Use of dengue NS1 antigen for early diagnosis of dengue virus infection. Southeast Asian J Trop Med Public Health. 2011; 42(3):562–9.
46) Hati A, Talukdar S, Bhattacharya N, Mukherjee H, Naskar R, Das G et al. Serological diagnosis of dengue in laboratory practice in Kolkata. Indian J Med Microbiol.. 2014 Jul-Sep; 32(3):277-280.
47) Golia S, Hittinahalli V, Karjigi KS, Reddy M, Kamath AS. Serodiagnosis of dengue using NS1 antigen, dengue IgM, dengue IgG antibody with correlation of platelet counts. IJ-AJIMS.2012 Nov; 1(2):112-117.

48) Khan SA, Dutta P, Topno R, Soni M, Mahanta J .Dengue Outbreak in a Hilly State of Arunachal Pradesh in Northeast India. The Scientific World Journal. 2014; 2014:1-6.
49) Chakraverti T, Malkappa S, Saileea. K. Comparision of Diagnostic efficacy of NS1 antigen based Immunochromatographic test with Immuno-sorbent assay as its role in detection of early Dengue infection.J of Evolution of Med and Dent Sci. 2012 Dec; 1(6):1066-1070.
50) Shu P, Huang J. Current Advances in Dengue Diagnosis. Clin Diagn Lab Immunol. 2004 Jul; 11(4):642-650.

51) Kao C-L, King C-C, Chao D-Y, Wu H-L, Chang G-JJ. Laboratory diagnosis of dengue virus infection: current and future perspectives in clinical diagnosis and public health. J Microbiol Immunol Infect. 2005; 38(1):5-16.

52) Perng G, Chokephaibulkit K. Immunologic hypo- or non-responder in natural dengue virus infection. J Biomed Sci. 2013; 20(1):34-39.
53) World Health Organization (WHO).Global Strategy for Dengue Prevention and Control, 2012–2020.Geneva: WHO Press; 2012.p1-43.
54) Shu P, Chen L, Chang S, Yueh Y, Chow L, Chien L et al. Comparison of Capture Immunoglobulin M (IgM) and IgG Enzyme-Linked Immunosorbent Assay (ELISA) and Nonstructural Protein NS1 Serotype-Specific IgG ELISA for Differentiation of Primary and Secondary Dengue Virus Infections. Clin. Diagn. Lab. Immunol. 2003 Jul; 10(4):622-630.
55) Chan K-S, Chang J-S, Chang K, Lin C-C, Huang J-H, Lin W-R et al. Effect of serotypes on clinical manifestations of dengue fever in adults. J Microbiol Immunol Infect. 2009; 42:471-478.
56) Lin C, Huang Y, Shu P, Wu H, Lin Y, Yeh T et al. Characteristic of Dengue Disease in Taiwan: 2002-2007. Am. J. Trop. Med. Hyg.2010; 82(4):731-739.
57) Hung N, Lei H, Lan N, Lin Y, Huang K, Lien L et al. Dengue Hemorrhagic Fever in Infants: A Study of Clinical and Cytokine Profiles.JID.2004 Jan; 189(2):221-232.
58) Alcon S, Talarmin A, Debruyne M, Falconar A, Deubel V, Flamand M. Enzyme-Linked Immunosorbent Assay Specific to Dengue Virus Type 1 Nonstructural Protein NS1 Reveals Circulation of the Antigen in the Blood during the Acute Phase of Disease in Patients Experiencing Primary or Secondary Infections. J of Clin Microbiol. 2002 Feb; 40(2):376-381.
59) Mahapatra D, Sarangi G, Mahapatra A, Paty B, Das P, Chayani N. NS1 Antigen Capture ELISA an Effective Method for Diagnosis of Early Dengue Infection - Report of an Outbreak at Angul District, Odisha, India. J Clin Diagn Res. 2014 Aug; 8(8): DC08–DC10.
60) Mishra N, Tripathi S, Khare V, Islahi S, Gulrez M, Shukla P et al.Comparison of NS1 Antigen and Antibody Detection Method in Early Diagnosis of Dengue Infection. IIJSR.2013; 4(1):2663-2668.
61) Palanivel H ,Nair S, Subramaniyan A, Ratnam P, Kanungo R,. Dengue virus infection: Need for appropriate laboratory tests for diagnosis and management of the condition in children during an outbreak. Indian J Pathol Microbiol.2015 Jul-Sept;58(3):328-331.
62) Naz A, Zahid D, Murky SN, Nadeem M, Sil BK, Shamsi ST. Evaluation of efficacy of various immunochromatographic rapid tests for dengue diagnosis. Pak J Med Sci.2014;30(1):166-171.