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Original Research Article

STUDY OF THE EPIDEMIOLOGY OF DENGUE VIRAL INFECTION AND COMPARISON OF THE EFFICACY OF RAPID TEST VERSUS ENZYME LINKED IMMUNOSORBENT ASSAY METHOD FOR DETECTION OF NS1, IGM AND IGG ANTIBODIES

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ABSTRACT

Background: A rapid, reliable, efficient, accurate and definitive laboratory diagnosis of Dengue infection, in first few days of clinical symptoms; helps in early confirmation of infection, helps in initiating appropriate management, reduces incidence of complications, helps in surveillance activities for outbreak control, aids in study of the pathogenesis of infection and in performing clinical trials. **Objective:** To assess and correlate sensitivity, specificity and diagnostic capability of immune chromatographic test with ELISA in diagnosing Dengue infection by detection of NS1 Antigen, IgM and IgG antibodies.
Materials and Methods: Prospective study was done on blood samples sent to microbiology laboratory from clinically symptomatic patients on 103 blood samples. Blood samples were drawn from febrile patients who were clinically symptomatic for Dengue infection. Around 5ml of venepuncture blood was collected using aseptic precautions. These samples were first tested for Dengue infection using commercially available rapid point-of-care tests.
Results: Mean age was 25.5years. Maximum number of patients are in 21-25 Years. Males=66% Females=34%. Mean duration of fever=4.363 days. Majority were diagnosed within 6 days of fever (96 cases). Most with platelet counts 20,000-40,000 were transfused platelets. 9 patients had presence of all 3 clinical signs–hepatomegaly, splenomegaly and ascites.
Conclusion: Study showed 96.67% sensitivity, 80.64% specificity, 90.63% PPV and 92.59% NPV for NS1 Antigen detection. This indicates that rapid kit had a good correlation with ELISA and can be used for early detection of Dengue infection in acute phase samples.
Key words: epidemiology, dengue, viral infection, antibody.

INTRODUCTION

Dengue infection is a rapidly emerging, fast spreading, arthropod - borne flaviviral infection; spread by mosquito vectors. This disease has re-emerged, in the last 50 years as an important febrile infection with increasing geographic expansion to new countries, causing major public health consequences. The disease is endemic in more than 100 tropical &sub-tropical countries of the world, especially in South-East Asia. The principal vector is Aedes aegypti mosquito, but recently Aedesalbopictus, Aedespolynesiensis & several species of Aedes scutellaris complex are identified.[1] According to WHO statistics, approximately 3.6 billion people live in Dengue endemic countries in the world, with an annual incidence of Dengue fever (DF) estimated globally to be between 50 -100 million cases. Among those infected, approximately 2.5 – 5 Lakh cases have complications like Dengue haemorrhagic fever (DHF) & Dengue Shock syndrome; with around 24,000 deaths commonly

seen in children. Nearly 75% of the current global disease burden is estimated to be in Asia, with India being one of the seven identified countries with hyper-endemic seasonal occurrence of the disease. The urban region case-fatality rates are 3-5% as per national statistics and all the four serotypes of Dengue virus are reported from India.[2]

Dengue virus has four distinct serotypes (DEN-1, 2, 3 and 4) but recently a fifth serotype; genetically different from the rest has been isolated. Recovery from infection by one serotype provides lifelong immunity against the same serotype but produces cross-immunity to other serotypes due to genetic similarity. Subsequent infections by other serotypes increase the risk of developing severe complications like DHF & DSS.[3]

Most infected patients usually are asymptomatic; few with severe infection and a small percentage develop complications like Dengue haemorrhagic fever (DHF), Dengue shock syndrome (DSS) which can be fatal. The clinically symptomatic patients initially present with febrile illness associated with symptoms like - malaise, headache, retro - orbital pain, body pain, joint pain, weakness and rash (as per WHO criteria). These symptoms overlap with other viral and bacterial infections like Chikungunya, Japanese encephalitis, leptospirosis, typhoid fever, scrub typhus, urinary tract infections etc., making accurate laboratory diagnosis necessary to avoid inappropriate treatment of patients.[4]

Dengue virus, though isolated in India in 1944, scientific studies addressing various problems of the disease are carried out only in few centres in India. A rapid, reliable, efficient, accurate and definitive laboratory diagnosis of Dengue infection, in the first few days of clinical symptoms; helps in early confirmation of infection, helps in initiating appropriate management, reduces incidence of complications, helps in surveillance activities for outbreak control, aids in study of the pathogenesis of the infection and in performing clinical trials.[5]

The laboratory tests routinely done, usually involve combination of tests which help to improve the sensitivity and specificity of diagnosis and depends on the availability of laboratory facility for the tests. The diagnostic methods currently available are: Virus isolation from acute-phase sera; Antigen detection by serological methods; Detection of antibodies by serological methods like ELISA; Viral nucleic acid (RNA) detection by molecular methods like reverse transcriptase PCR (RT-PCR).[6]

Due to the high cost, requirement of trained staff with technical expertise, the requirement of specialised equipment and time before positivity in the gold standard tests, many companies have developed rapid diagnostic tests based on the principle of immunochromatography which are available in the market. These kits developed are point-of-care immunochromatographic tests (RDT's) which are rapid, easy to perform, detect both antigen and antibodies, are cost effective; give results in 15-20 minutes, do not require trained hands, while being used especially in remote settings in the developing countries. But these kits need to be validated and compared with the available gold standard tests before being used for diagnosis.[7]

In the defervescence period of infection, patients usually have significant thrombocytopenia (platelets 20% less than normal), leucocytopenia (Total count < 4,000 cells/mm3), signs of plasma leakage like ascites, pleural effusion, raised SGOT; SGPT levels, hypoproteinaemia and clinical signs like severe continuous abdominal pain, restlessness, somnolence, persistent vomiting, sudden reduction in temperature, profuse perspiration, malaise, haemorrhagic manifestations etc.[8]

In this study, commercially available rapid Dengue diagnostic kit was compared statistically with accepted standard test, ELISA to detect the presence of Dengue NS1 antigen, IgM/IgG antibodies in acute phase serum. The association of platelet count, leukocyte count, with Dengue infection & the duration of fever were correlated, which indicate disease severity and aid in early diagnosis. The presence of primary or secondary infection using IgM/IgG ratio was established.

MATERIALS AND METHODS

A prospective study was done on blood samples sent to the microbiology laboratory from clinically symptomatic patients (as per inclusion criteria) admitted in Shadan Institute of Medical Sciences, Hyderabad over a period of 1 year 6 months. This study was done on 103 blood samples received in the Microbiology laboratory for diagnostic confirmation of Dengue infection. The blood samples were drawn from febrile patients who were clinically symptomatic for Dengue infection (as per WHO criteria), admitted in the Departments of Medicine, Paediatrics, Emergency Medicine, OBG, Orthopaedics etc. at Shadan Institute of Medical Sciences, Hyderabad. Serum obtained by centrifuging from above blood samples was subjected for the following tests to confirm Dengue infection.

Positive controls, Negative controls and calibrators provided by the manufacturer along with the kits were tested for credibility and validity of the kits.

Inclusion Criteria

- Patients with febrile illness who are clinically symptomatic for Dengue infection (as per WHO criteria) of all ages and both sexes are included in the study.

Exclusion Criteria

- Patients who fail to give consent for the serological and molecular diagnosis
- Patients with autoimmune diseases
- Serum samples which are icteric or exhibiting haemolysis, showing lipaemia, microbial growth

Ethical clearance was given by the Scientific and Ethical Committee at SIMS; Hyderabad and the

procedures were carried out in accordance with the set protocols.

The demographic data i.e. the patient details taken for the above study were - Name, Age, Sex, Father / Guardian's name, Address with phone number, Occupation, admission & discharge date and health care provider name were collected and tabulated.

The clinical data regarding the duration of fever, details of the illness, symptoms, and signs of Dengue fever were tabulated and analysed.

The clinical laboratory data from haematology department: platelet count and leukocyte count were collected, tabulated and analysed.

Around 5ml of venepuncture blood from patients included in the study was collected using aseptic precautions and sent immediately to the Microbiology laboratory in the red capped collection vials without anticoagulant. The blood samples were allowed to clot at room temperature (20 - 25oC) for 30min and then centrifuged as per CLSI protocols to separate the serum in supernatant. The serum thus separated was stored sterile plastic vials for testing and further use. The samples were refrigerated at appropriate temperatures (2-8oC, -20oC and -80oC) for serological and molecular testing respectively.

These samples were first tested for Dengue infection using commercially available rapid point-of-care tests by immunochromatography using SD BIOLINE Dengue Duo kits as described below. Those samples which were positive for Dengue infection {NS1 Antigen (and/or) IgM/IgG Antibodies positive} were taken for the study.

These above positive samples were tested for presence of NS1 Antigen, IgM/IgG Antibody by ELISA (which is taken as standard) for confirmation using commercially available PAN BIO Dengue Early ELISA, Dengue IgM Capture ELISA and Dengue IgG Capture ELISA.

The results were tabulated and compared with rapid test. The ELISA results were used to calculate the IgM/IgG ratio to identify primary (ratio >1.2) or secondary (ratio <1.2) Dengue virus infection.

All tests in this study were carried out in accordance with the manufacturer's instructions. Appropriate precautions and warnings mentioned by the manufacturer were followed.

1) SD BIOLINE Dengue Duo (Dengue NS1 + Ab Combo): Standard Diagnostics Inc, Korea.

2) DENGUE EARLY ELISA: PANBIO, Inverness Medical, Brisbane, Queensland, Australia.

3) DENGUE IgM CAPTURE ELISA: PANBIO, Inverness Medical, Brisbane, Queensland, Australia.

4) DENGUE IgG CAPTURE ELISA: PANBIO, Inverness Medical, Brisbane, Queensland, Australia.

Statistical Analysis:

Data collected was analysed statistically using appropriate statistical tests. The Percentage, mean, sensitivity, specificity, PPV, NPV with 5% level of significance was calculated for the data using statistical software. The results were depicted in pictorial form Using graphs and tables for better understanding. The following assumptions were made for analysing the data:

RESULTS

Table 1: Prevalence of dengue viral fever: age wise distribution

AGE IN YEARS	NUMBER OF PATIENTS	PERCENTAGE (%)
1–5	5	4.8
6--10	7	6.7
11--15	10	9.7
16-20	15	14.5
21-25	18	17.4
26-30	15	14.5
31-35	10	9.7
36-40	10	9.7
41-45	3	2.9
46-50	5	4.8
51-55	3	2.9
56-60	2	1.9
Total	103	

Mean age of 25.5years, Median of Age = 24 years.

Maximum number of patients are in the of age group of 21-25 Years.

Table 2: Prevalence of denv: gender distribution

Gender	Number	Percentage
Male	68	66.1 %
Female	35	33.9 %

Males =66% Females = 34 % Male: Female ratio = 1.94

Table 3: Details of duration of fever in patients studied

Duration Of Fever(Days)	Number	Percentage
1 – 3	25	24%
4 – 6	71	69%

7 - 10	7	7%
Grand Total	103	

- Mean duration of fever = 4.363 days.
- Median duration of fever = 4 day.
- Maximum patients had duration of fever of 4 to 6 days = 71(69%)
- 24% of patients have Duration of fever of 1 to 3 days.
- Majority of the patients were diagnosed within 6 days of fever in 93% (96 cases)

Table 4: Details of clinical signs, tourniquet test and platelet transfusion in patients studied

	TOURNIQUET TEST	HEPATOMEGALY	SPLENOMEGALY	ASCITIS	PLATELET TRANSFUSION
Positive	32	33	11	33	37
Negative	71	70	92	70	66

The clinical signs like hepatomegaly, splenomegaly, ascites, and tourniquet test and platelet transfusion have been analysed.
Tourniquet test positive patients – platelet count = 6,000 to 40,000 cells/mm3
Most patients with platelet counts 20,000 to 40,000 were transfused platelets.
9 patients had presence of all 3 clinical signs – hepatomegaly, splenomegaly and ascites.

Table 5: Details of distribution of platelet count in patient studied

Platelet Range	Total	Percentage
5000-15000	15	14.5
15000-50000	43	41.7
50000-100000	29	28.1
100000-150000	12	11.6
150000-400000	4	3.8
>400000		0

15 (14.5%) patients had a platelet count <15,000; indication of transfusion.
Maximum patients 43 (41.7%) had platelet count between 15,000- 50,000.
Only 3.8% patients had normal platelet count.

Table 6: Details of Distribution of Total Leucocytic Count

Total leucocyte count	Number
< 4000 cells/mm^3	41
4000- 11000cells/mm^3	58
>11000 cells/mm^3	3

- 41 Subjects have Leucocyte count < 4000 cells/mm3, due to immune mechanism of Dengue infection.
- 58 subjects have a normal count.
- 4 patients had a raised leucocyte count, due to any bacterial infections.

Table 7: Details of Comparison of Rapid Diagnostic Kit with Elisa

SD NS1 VERSUS ELISA NS1			
SD/ELISA	**POSITIVE**	**NEGATIVE**	**TOTAL**
POSITIVE	64	8	72 (SD)
NEGATIVE	3	28	31
	67 (ELISA)	36	103

SD IgM VERSUS ELISA IgM			
SD/ELISA	**POSITIVE**	**NEGATIVE**	**TOTAL**
POSITIVE	59	0	59 (SD)
NEGATIVE	7	37	44
	66 (ELISA)	37	103

SD IgG VERSUS ELISA IgG			
SD/ELISA	**POSITIVE**	**NEGATIVE**	**TOTAL**
POSITIVE	53	0	53 (SD)
NEGATIVE	6	44	50
	59 (ELISA)	44	103

The statistical analysis is as below.

Table 8: Comparative table showing the statistical analysis

S.NO	STATISTICAL TESTS	SD NS1	ELISA NSI	SD IgM	ELISA IgM	SD IgG	ELISA IgG
1	SENSITIVITY	0.9667	96.6%	0.896	89.66%	0.9038	90.38%
2	SPECIFICITY	0.8064	80.6%	1	100%	1	100%
3	POSITIVE PREDICTIVE VALUE	0.9063	90.6%	1	100%	1	100%
4	NEGATIVE PREDICTIVE VALUE	0.9259	92.5%	0.846	84.62%	0.8864	88.64%
5	PREVALENCE	0.6593	65.9%	0.637	63.74%	0.5714	57.14%
6	PRE TEST ODDS	1.9354	-	1.757	-	1.3333	-
7	POST TEST ODDS	3.2931					
8	LR POSITIVE	4.9944					
9	LR NEGATIVE	0.0413	-	0.103	-	0.0962	-
10	POST TEST PROBABILITY	0.7676	-	NA	-	NA	-

Table 9: Determination of primary and secondary dengue infection by IgM/IgG Ratio in The Subjects

Classification Based On Elisa Result	Number
Primary Infection	52
Secondary Infection	51

37 patients with NS1 Ag positive –has Primary infection
41 patients had IgM Antibody positive have Primary infection
27 patients having both NS1 Ag and IgMAb positive have primary infection.

DISCUSSION

In this study, patients were selected without any age and gender preferences.[21] As indicated in table 1: minimum age of patients was 1 year and maximum age is 58 years. The mean age was 25.71 years and median age was 24 years. Maximum numbers of patients were in the age group of 21-25 years (18.68%). This study indicates that the young adults are the most affected by Dengue fever similar to other studies. The children and adolescence are found to be predominantly affected with primary and secondary infection in Southeast Asia, including India due to low susceptibility in adult population because of multitypic immunity.[9] Another reason could be that paediatricians gain more experience in the management of Dengue virus infections and request tests only for children whose symptoms make a clinical diagnosis highly uncertain, while physicians treating adults request tests for patients with clinical diagnosis of Dengue virus infection.[10]

The gender variation of patients in the study is as shown in table 2: The distribution among patients was Male 66 % & Female 34%. Male: Female ratio = 1.94: 1. Males had higher prevalence of probable Dengue infection than females which is in accordance with many other studies as indicated. Male preponderance in young adult age group may indicate greater chance of infection at work sites. Lower infection rates in females of Asian community might be attributed to lower reporting rate; females remain at home and are less exposed to vector mosquito. The lower disease incidence among women may be a statistical artefact. Hence well-designed and targeted studies are required to confine both biological and social factors that confer disease patterns in a community.[9]

The duration of fever is an important indicator for early diagnosis of Dengue fever, in detecting primary or secondary infection on comparison with serological tests (correlating the appearance of NS1Antigen, IgM/IgG Antibodies). In this study, duration of fever (DOF) ranges from 1 to 10 days, mean DOF being4.363 days and median is 4 days. Maximum number of patients had DOF between 4-6 days (69%) which correlated with the other studies.[11,12] This study indicates that majority of the patients were confirmed to have the disease within 6 days of fever (93%) by the tests used indicating that RDT used was efficient in rapid diagnosis helping in early management and reducing complications. NS1Antigen positive patients had DOF of 2 to 5 days, IgM Antibody positive had DOF of 4 - 5 days and IgG Antibody had DOF of 2 to 7 days.

The study subjects showed following clinical features: headache, chills, nausea, vomiting, retro-orbital pain, arthralgia, joint pain, myalgia, malaise, flushed face, lethargy, abnormal bruising, lymphadenopathy, jaundice, bleeding from the nose and other orifices. These symptoms and signs were associated with serological positivity, platelet count & duration of fever. The viral multiplication in the liver and the viremia are seen to have an important effect in the clinical manifestations. The patients with positive Tourniquet test had a platelet count between 6,000 to 40,000/mm.3 18 patients had hepatomegaly and ascites, 8 patients had hepatomegaly and splenomegaly. Studies done by Butt N et.al and Ahmed S et.al compared the common signs and symptoms, showed mucosal bleeding manifestations in 34.6%; hepatomegaly in 56.7%, 0.5% and splenomegaly in 12.5%, 11.2% respectively; lymphadenopathy & pleural effusion and ascites in 7.6%.[13,14]

In resource poor health care system, along with lab tests, platelet count is another accessory test done in peripheral laboratories which has an importance in management. In majority of patients', thrombocytopenia is transient and asymptomatic, but in significant number of cases there is bleeding manifestations. A regular platelet count testing, every 8th hourly especially after platelet transfusion is essential. In our study as in table 7: 87 patients had significant thrombocytopenia; 14.5% patients had a platelet count <15,000; 41.7% had a platelet count of

15,000 - 50,000 and many patients were transfused platelets. 28.1% had a platelet count between 50,000 -1 lakh, were monitored for drop in platelets and 15.4% patients had a platelet count > 1 lakh which correlated with many studies done. The presence of petechial, purpura, ecchymosis, epistaxis, bleeding gums, positive tourniquet test correlated with those who had significant thrombocytopenia. Similar findings were reported by other studies.[9,15] As many diseases, clinical conditions like viral infections, collagen vascular diseases, idiopathic and drug use cause thrombocytopenia, complete dependence on platelet count for diagnosis is not accurate.

The raised leukocyte count signifies the presence of infection, especially of bacterial origin in the patients but many studies have shown a low leukocyte count (< 4000 cells/mm3) in Dengue fever due to immune mechanism. This study showed that 41 subjects had low leukocyte count (< 4,000 cells/mm3), 58 subjects had normal counts and 3 had raised leukocyte count (> 11,000 cells/mm^3) which may indicate infection of bacterial origin.

Dengue illness appears similar to other febrile illnesses in its early stages; hence diagnosis is often delayed or confused with other illnesses. However, the speed and accuracy of diagnosis must be balanced against test cost and availability, especially in developing countries. This study was mainly done to establish that the commercially available immunochromatographic test can be used as an alternative to the accepted standard tests like ELISA as they have a high sensitivity and specificity. The ability of the rapid kit to correctly identify the presence of NS1 Antigen in the study subjects of Dengue fever with the acute phase serum samples indicated by the sensitivity is 96.67%, a very high value. The ability of the rapid kit to correctly identify the absence of NS1 Antigen in the acute phase serum samples is 80.64%. This shows that the specificity of the test is low and needs to be evaluated further. The chance that the positive test result indicates the presence of the disease condition indicated by Positive predictive value is 90.63%. This indicates that the test kit helps for better confirmation of the disease. The chance that the negative test result indicates the absence of the disease condition indicated by Negative predictive value is 92.59%. This kit can rule out patients as not having early infection with good accuracy. The Likelihood ratio positive where the positive test result indicates that a subject is 4.9944 times more likely to be truly positive than negative as compared to the standard ELISA test in case of rapid kit. The Likelihood ratio negative which indicates that with a negative result in the test done, a subject is 0.9587 times likely to be positive than negative to have the disease as determined by ELISA test. This value is low and further study investigations need to be done. Thus, the rapid kit had a high sensitivity, PPV and NPV in detecting NS1 Antigen in the samples and the detection of NS1 Antigen helps in detecting infection in early and acute phase of disease.

The ability of the rapid kit to correctly identify the presence of IgM antibody in the subjects of Dengue fever from acute phase samples, indicated by sensitivity of the test is 89.66%. This parameter indicates a significantly high value, but further sampling investigations need to be done before the actual performance of the kit can be compared as the sensitivity is below 90%. The specificity of the test indicating the ability of the rapid kit to correctly identify the absence of IgM antibody in the samples is 100%. This indicates that the test kit is very efficient in identifying individuals without the disease. The Positive predictive value indicating the chance that a positive test result will indicate the presence of the disease condition is 100% indicating that healthy subjects were not detected as having Dengue infection. The Negative predictive value indicating, the chance of a negative test result to indicate the absence of the disease condition is 84.62%. There seems to be a significant drop in the NPV and may need further statistical investigations to obtain a strong, evidence to comment. The Likelihood ratio positive where the positive test result with the kit indicates that a subject is infinite times more likely to be truly positive than negative as compared to the standard ELISA test in case of rapid kit. The Likelihood ratio negative which indicates that with a negative result, a subject is 0.8966 times likely to be positive than negative to have the disease as determined by ELISA test. A higher value would have a better statistical significance. The rapid kit is 100% specific, has 100% PPV and a high NPV, which indicates that the rapid kit can detect IgM Antibody in infection better in acute phase of disease.

The ability of the rapid kit to correctly identify the presence of IgG antibody in the serum samples of Dengue fever indicated by sensitivity of the test is 90.38%. There is a significant drop in the sensitivity of the kit in detecting presence of old Dengue infection and further sampling investigations need to be done. The specificity of the test indicating the ability of the rapid kit to correctly identify the absence of IgG antibody in the serum samples is 100% which indicates that the test kit is very efficient in identifying individuals without the disease. The chance that a positive test result will indicate the presence of the disease condition indicated by the Positive predictive value is 100%. The Negative predictive value which indicates the chance of a negative test result indicating the absence of the disease is 88.64%. There seems to be a significant drop in the NPV but this may not give a strong evidence to comment on the efficiency of the kit. The Likelihood ratio positive here indicates that a subject is infinite times more likely to be truly positive than negative as compared to the standard ELISA test in case of rapid kit. The Likelihood ratio negative which indicates that a negative result in the test done using rapid kit, a subject is 0.0038 times likely to be positive than negative to have the disease as determined by ELISA test. The rapid kit performed better in the sensitivity, specificity, PPV and NPV

and can be used for detecting presence of previous infection by Dengue Virus. From the above test parameters, the statistical analysis of the rapid diagnostic kit with respect to the standard ELISA test; indicate that the RDT can be used as an alternative to the ELISA test kit in laboratories especially in screening and detecting the infection but more samples need to be tested before giving a final comment. Several studies,[16,17] have been done using the rapid diagnostic kits which have been introduced in the market.

The high sensitivity and specificity of ELISA test as demonstrated by many studies for diagnosing Dengue infection, which helps us to accept ELISA as a standard test for diagnosis of the infection. In a study done by Shankar SG et.al, RT-PCR detected 14 Dengue cases whereas NS1 &IgM ELISA detected 19 cases including all 14 RT-PCR positive samples.[18]

Infection by one serotype induces lifelong immunity, but provides only a short and transient protection against further infection by the other serotypes. Some studies indicate re-infection by same serotype can also lead to complications. The changes in the serotype in each epidemic can lead to development of complications of Dengue in previously infected subjects. Hence early detection of Dengue virus and prevalent serotype helps in minimizing disease burden and controlling disease spread.

The presence of primary or secondary Dengue infection can be determined by: presence of NS1 Antigen, IgM and IgG antibodies in the patient. IgM/IgG ratio using ELISA is more accurate in determining primary or secondary infection. Haemagglutination test was used initially but not used nowadays. Based on IgM: IgG ratio, in our study we found 52 patients had primary infection and 51 patients had secondary infection. Among 52 primary infection patients, we found 37 patients also had NS1 antigen positive in ELISA, and 41 patients had IgM positive, Predicting the importance of NS1 along with IgM: IgG ration in differentiating primary and secondary dengue infections.

In this study there was only 1 patient who had a Widal test positive indicating typhoid fever and all others were negative for Typhoid fever, Malaria, Chikungunya infections. A study done by Karia J et.al showed 30 had co-infection of Malaria and 4 were positive for Widal test.[19] In a study done by Saxena P et.al, there was no cross-reaction with closely related members of flavivirus (Japanese encephalitis, West Nile, Yellow fever) and alpha virus.[20] a study done by Guru Kumar KR et.al showed no co-infection with Japanese Encephalitis, West Nile fever, Chikungunya, Leptospira, Plasmodium vivax, Plasmodium falciparum and Rickettsia infection. In a study done by Hang VT et.al, there was no co-infection with enteric fever, malaria, Japanese encephalitis and leptospirosis.[12]

The periodicity of occurrence of Dengue infection can be postulated due to the climate and environmental aspects that play a critical role in the distribution and Copyright and confidential. Prevalence of both Dengue virus and its vectors. This seasonal outbreak of the disease transmission emphasizes the need for appropriate vector control measures to be implemented into full swing during water stagnation periods; i.e. after the initial bouts of rainfall, at the end of monsoon and post monsoon months to reduce the case incidence.[9] In this study most of the patients were infected during the monsoon and post monsoon season especially during July to November with most patients infected during September to November.[21] This seasonality was seen in many studies, as in study done by Chakravarti Aet.al, where 70 out of 85 serologically positive cases were reported during October – November. In the study done by Advani S et.al, most patients were infected during September – November 2008. The study done by Ukey P et.al reported that most of the patients in their study were infected during the month of November, followed by in October.[22]

One important investigation which needs to be done is the ultrasound of the abdomen and pelvis. Many studies have mentioned the importance of is investigation as an adjunct to clinical and laboratory profile in diagnosing Dengue fever or Dengue haemorrhagic fever and in predicting the severity of the disease as indicated by studies done by Venkatasai PM et.al and Colbert K et.al.[23,24] This study will be extended to involve more samples and determine the USG findings in relation to the disease.

CONCLUSION

The study showed 96.67% sensitivity, 80.64% specificity, 90.63% PPV and 92.59% NPV for NS1 Antigen detection. This indicates that the rapid kit had a good correlation with ELISA and can be used for early detection of Dengue infection in acute phase samples. The rapid kit for IgM Antibody had sensitivity - 89.66%, specificity - 100%, PPV - 100% and NPV - 84.62%. The rapid kit for IgG Antibody had sensitivity - 90.38%, specificity - 100%, PPV - 100% and NPV - 88.64%. The study showed that the specificity and PPV of the rapid kit for antibodies matched with ELISA and sensitivity, NPV were high indicating that the rapid kit was statistically competent to detect both acute and convalescent infection and can be used as an alternate to the standard tests for Dengue infection diagnosis. In this study, the younger age group especially males were affected. Most of the patients had mean duration of fever of 4.363Days. The clinical symptoms and signs like haemorrhagic manifestations, hepatomegaly, splenomegaly and ascites had correlation with serological diagnosis and platelet count. Patients with low platelet counts were transfused platelets depending on the clinical condition. Most of the patients were infected during the months of July to November. All patients with IgMAb positive had primary infection; IgG Ab positive had secondary infection. 18 patients with NS1Ag positive had

secondary infection and could be more prone to complications.

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