International Journal of Pharmaceutical Science Invention
ISSN (Online): 2319 – 6718, ISSN (Print): 2319 – 670X
www.ijpsi.org || Volume 3 Issue 12 || December 2014 || PP.11-16

Evaluation of Rapid ICT in comparison with MAC-ELISA in diagnosis of dengue fever at a tertiary care hospital, South India.

Tabasum Begum M[1], Dr. Sumana MN[2], Dr. Basavana Gowdappa H.[3]
[1](Department of Microbiology, JSS Medical College/ JSS University, India)
[2](Department of Microbiology, JSS Medical College/ JSS University, India)
[3](Department of Medicine, JSS Medical College/ JSS University, India)

ABSTRACT : *Dengue has emerged as a major public health concern throughout India. It is the most common mosquito-borne viral disease of humans. This study aims to determine demographic, clinical and laboratory investigations along with the disease outcome of all the suspected cases of dengue fever and comparison of two commercial tests routinely useful in diagnosis of dengue fever.*

MATERIALS AND METHODS*: A total of 228 serum samples from patients with suspected dengue infection were included and the study was undertaken at a tertiary care hospital from July 2013-October-2013. All samples were subjected to rapid Immunochromatographic card test (ICT) and IgM-Capture ELISA (MAC-ELISA). Result: The sensitivity and specificity of ICT was 66.24% and 90.14% while it was 93.69% and 54.70% for MAC-ELISA. Platelet count, Aspartate Aminotransferase (AST) and Alanine transaminase (ALT) levels of all the dengue positive cases and healthy controls were recorded and comparison showed that these parameters are statistically significant with p-value <0.0001. Conclusion: Dengue NS1 antigen detection through immunochromatographic rapid card test along with MAC-ELISA proves to be more sensitive in the early diagnosis of dengue virus infection.*

KEYWORDS : *ALT, AST, Dengue Fever, ICT, MAC-ELISA.*

I. INTRODUCTION

Dengue is the most rapidly spreading mosquito-borne viral disease in the world. It is commonly found in tropical and subtropical countries. Dengue Fever (DF) is caused by an arbovirus and spread by *Aedes* mosquitoes (Sumana MN *et al; 2014*). In last 50 years, incidence has increased 30-fold with increasing geographic expansions to new countries and in the present decade, from urban to rural settings (WHO; 2009). An estimated 50 million dengue infections occur annually and approximately 2.5 billion people live in dengue endemic countries (Guzman M.G ; 2004). Dengue in India was first reported during 1956 from Vellore district in Tamil Nadu. The first Dengue Hemorrhagic fever (DHF) outbreak occurred in Calcutta (West Bengal) in 1963 with 30% of cases showing hemorrhagic manifestation. All the four serotypes that is Dengue 1, 2, 3 and 4 have been isolated in India. Recurring outbreaks of DF/DHF have been from various states namely Andhra Pradesh, Delhi, Goa, Haryana, Gujarat, Karnataka, Kerala, Maharashtra, Rajasthan, Uttar Pradesh, Pondicherry, Tamil Nadu, West Bengal and Chandigarh (Guidelines for clinical management of Dengue fever, Dengue Hemorrhagic fever and Dengue shock syndrome; 2008; Nivedita G *et al; 2012*). Dengue virus infection continues to be the public health concern in Karnataka and neighboring states (Epidemiological investigations on dengue virus infections in Karnataka and neighboring areas; Annual Report 2004-2005). Dengue is one of the most important mosquito-borne diseases in the world but still remains very much under reported, especially in developing countries where diagnostic facilities are inadequate. Most patients with dengue infections are asymptomatic. The severe forms of dengue fever (DF) are dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Early symptoms of dengue fever mimic other diseases often prevalent in areas where it is endemic, such as malaria and leptospirosis (Satish N *et al; 2003)*. Thus, a rapid differential diagnosis is crucial for proper patient care.

The precise diagnosis of dengue infection can be achieved through viral isolation, viral RNA detection through RT-PCR or by detecting dengue specific antigen or antibodies (Shrivastava A *et al*; 2011). As the first two methods are time consuming, costly and not within the reach of even most of the tertiary care hospitals, its diagnosis is based on the detection of dengue specific antibodies and/or NS1 antigen (Kumaraswamy V *et al;* 2011). According to the guidelines of National Vector Borne Disease Control Programme (NVBDCP), IgM antibody capture ELISA should be considered as diagnostic test for dengue infections which will help in early diagnosis of dengue (Directorate of National Vector Borne Disease Control Programme; 2008) . However, most

of the private hospitals and several diagnostic laboratories are using various rapid immunochromatographic tests (ICT) to detect the dengue specific antibodies and/or antigen (WHO; 1997). There was an outbreak of dengue infection in and around Mysore district of Karnataka in the year 2013 (July 2013-October 2013) and hence, the study was undertaken to prospectively analyze the demographic, clinical and laboratory features of suspicious cases of dengue fever and evaluation of ICT and MAC-ELISA used for the diagnosis of dengue to establish an accurate and early diagnosis of acute dengue infection at a tertiary care hospital in Mysore, Karnataka.

II. MATERIALS AND METHODS

A total of 228 serum samples obtained from clinically suspected cases of dengue infection were tested in Microbiology Department of JSS Medical College and Hospital, Mysore from July 2013 to October 2013. Since our laboratory works around the clock, the samples were tested immediately for NS1 antigen, IgG and IgM antibodies by rapid visual immunochromatography-based test (ICT (EZDX Dengue Combo Ag-Ab) from Advy chemicals Pvt.Ltd. Thane, Mumbai for dengue NS1, IgG and IgM as per manufacturer's instructions. All the samples were then subjected to Dengue IgM-capture ELISA by Inbios IgM antibody capture MAC-ELISA, Seattle, USA. Clinical and laboratory manifestations of all the dengue seropositive cases and 50 dengue seronegative cases which served as controls were recorded.

III. RESULTS AND DISCUSSION

Dengue specific antibodies were tested by IgM capture ELISA and ICT. Out of the 228 samples tested, 157 were positive by MAC-ELISA and 111 were found to be positive for dengue infection by rapid ICT. Of these NS1 antigen alone was positive in 48, IgG was positive in 24, IgM was positive in 31 and both NS1, IgM parameters were positive in 08.The samples tested with rapid ICT and IgM capture ELISA and comparison of these tests showed that of the total 228 samples, 104 were positive and 64 were negative by both the tests. There were 53 samples which were positive by ELISA and negative by ICT and were considered as false positive. 7 (NS1 antigen positive) samples were positive by ICT and negative by ELISA test and were considered as false negative. The sensitivity, specificity, positive predictive values between the two tests are given in Table1.

TABLE 1: SENSITIVITY AND SPECIFICITY OF ICT AND MAC-ELISA

	Sensitivity	**Specificity**	**PPV**	**NPV**
ICT	**66.24%**	**90.14%**	**93.69%**	**54.70%**
MAC-ELISA	**93.69%**	**54.70%**	**66.24%**	**90.14%**

Many Immunochromatographic test devices for detecting dengue NS1, IgM and IgG antibodies are commercially available and many studies have evaluated their performances. In this study, the performance of ICT (EZDX Dengue Combo Ag-Ab) from Advy chemicals Pvt Ltd. Thane, Mumbai for dengue NS1, IgG and IgM) and MAC-ELISA (Inbios IgM antibody capture MAC-ELISA,Seattle,USA) was compared and the sensitivity of ICT was 66.24% and sensitivity of MAC-ELISA was 90.14% where as specificity of ICT obtained was 93.69% and specificity of MAC-ELISA was 54.70%.

Better sensitivity of IgM-capture ELISA in comparison to rapid ICT have been reported by Moorthy etal and Jayasimha et al (Moorthy M *et al;* 2009; Jayasimha VL *et al ;* 2012). Several other studies showed differences in sensitivity and specificity of ELISA and rapid tests and their difference might be due to the different principles of these assays (Palmer CJ; 1999).

In a study by Pramiladevi *et al*., a total of 66 probable dengue cases were selected. 16 cases were found to be positive for dengue rapid ICT, whereas 14 cases were found to be dengue positive by IgM capture ELISA. The sensitivity and specificity of rapid test along with positive predictive value and negative predictive value were deducted and compared with other studies. The study shows that the sensitivity of rapid card test is less but has a good specificity. In situations of epidemic, the card test can be used for screening but with the support of IgM capture ELISA. Highly suspicious cases should be subjected to investigations with higher sensitivity and specificity, though the results take little more time (Dr. Pramiladevi *et a ;* 2013).

In our study, the demographic profile of patients with dengue fever was recorded. Out of 228 patients with symptoms of dengue fever, 157 patients were found to be positive for dengue infection. Based on the degree of clinical manifestations, the patients were classified as Dengue fever (134) and Dengue fever with Thrombocytopenia where the platelet count is less than 1,00,000/ml (23) (National Rural Health Mission;

2012). Of these 104 were males while 53 were females, giving a ratio of (2:1). The age distribution is shown in Table 2. According to Age and sex wise distribution, there was more number of males in the age group of 15-35. (Figure1&2). This is one of the most extensive studies to report dengue fever along with the clinical and serological characteristics during the recent outbreak in and around Mysore district.

TABLE 2: AGE AND SEX WISE DISTRIBUTION

	Dengue Fever (DF) N=134		**Dengue Fever with Thrombocytopenia (DFT) N= 23**	
Age Group	**No. of Cases (%)**		**No. of Cases (%)**	
	Males	**Females**	**Males**	**Females**
0-15 (<3yrs)	**14 (10.44%)**	**6 (4.47%)**		
15-30	**40 (29.85%)**	**18(13.43%)**	**3 (13.04%)**	**2 (8.69%)**
30-45	**21 (15.67%)**	**13 (9.70%)**	**9 (39.13%)**	**3 (13.04%)**
45-60	**7 (5.22%)**	**5 (3.73%)**	**2 (8.69%)**	**1 (4.34%)**
>60	**6 (4.47%)**	**4 (2.98%)**	**2 (8.69%)**	**1 (4.34%)**

Figure 1: Age and Sex wise distribution of Dengue fever (n=134)

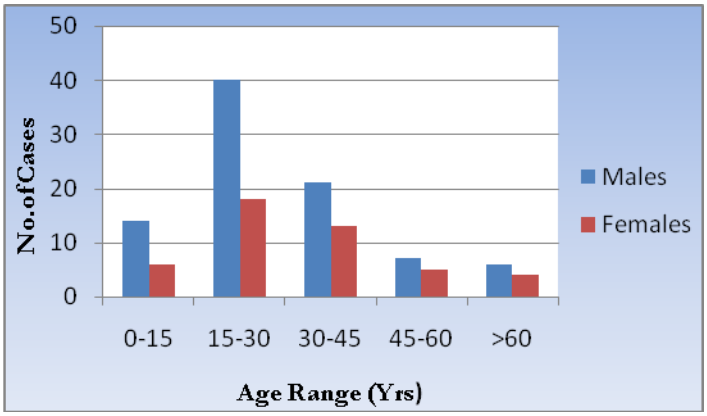
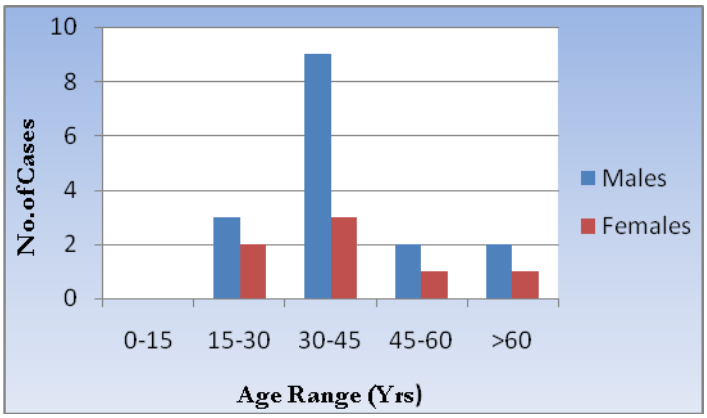


Figure 2: Age and **Sex wise distribution of Dengue fever with Thrombocytopenia (n=23)**



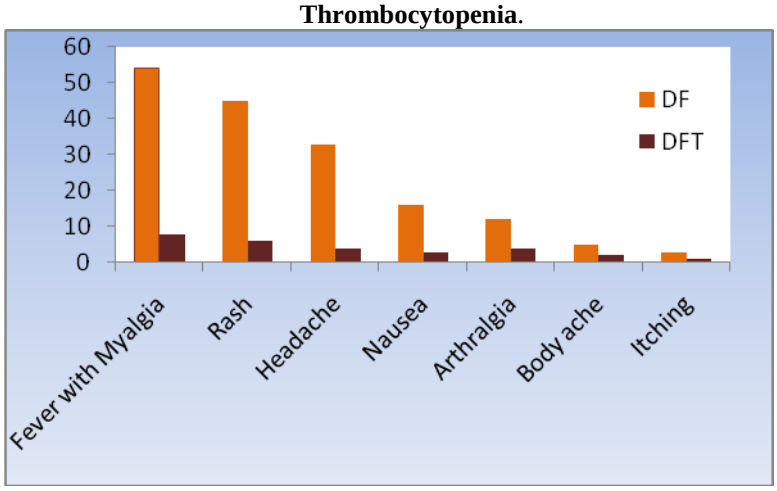
Clinical Manifestation observed in patients (DF, DFT) had fever along with myalgia (43.2%, 26.08%), rash (20.89%, 8.69%), headache (14.17%, 21.73%), nausea (7.46%, 17.39%), arthralgia (5.97%, 0%),

body ache (5.22%, 17.39%) and itching (2.98%, 8.69 %). The clinical manifestation of patients is shown in Table 3. Fever with myalgia, rash and arthralgia were more prevalent in DF patients whereas headache, nausea, bodyache and itching were more prevalent in patients with DFT (Figure 3).

Table 3: Clinical Manifestation of Patients

Signs and Symptoms	No. of Cases	
	Dengue Fever (DF)	Dengue Fever with Thrombocytopenia (DFT)
Fever with Myalgia	**54**	**8**
Rash	**45**	**6**
Headache	**33**	**4**
Nausea	**16**	**3**
Arthralgia	**12**	**4**
Bodyache	**5**	**2**
Itching	**3**	**1**

Figure 3: Clinical Signs and Symptoms of Dengue fever and Dengue fever with Thrombocytopenia.



Laboratory investigation showed that there was a 4 fold decrease in platelet count in DFT and 1 fold decrease in platelet count in DF (Figure 4). AST levels (IU/L) for DFT, DF & HC were found to be 196± 35.2; 69 ±17.96; 28.18± 3.2 respectively and ALT levels (IU/L) found were 171.29±38.18; 48.4±4.9; 28.39±3.32 respectively as shown in Table 4. An 8 fold increase in DFT and 3 fold increase in DF of AST level was observed in both the groups; 7 fold and 2 fold increase of ALT level was observed in both the groups respectively when compared to the healthy controls (Figure 5& 6).

Table 4: Comparison of Platelet count, AST level, ALT level in DF, DFT and HC.

	DF	DFT	HC	p-value	F-value
Group a: Comparison of platelet count ($X10^3$) Normal Range : 150-400	**119±4.5**	**56±15.18**	**150±3.2**	**0.0001**	**4572**
Group b: Comparison of AST level (IU/L) Normal Range : 10-34 IU/L	**196 ± 35.2**	**69±17.96**	**28.18±3.2**	**0.0001**	**3319.06**
Group c: Comparison of ALT level (IU/L) Normal level : 10-40 IU/L	**171.29±38.18**	**48.4±4.9**	**28.39±3.32**	**0.0001**	**2754.6**

Figure 4: Comparison of Platelet count of Dengue fever with Thrombocytopenia (DFT), Dengue fever (DF) and Healthy Controls (HC).

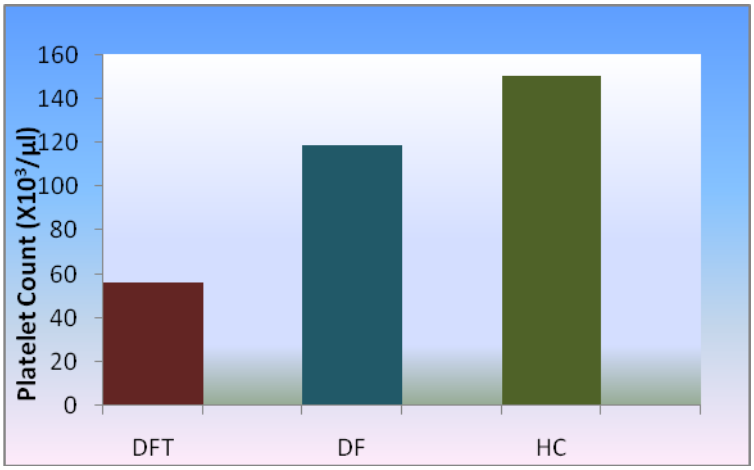


Figure 5: Comparison of AST level of DFT, DF and HC subjects.

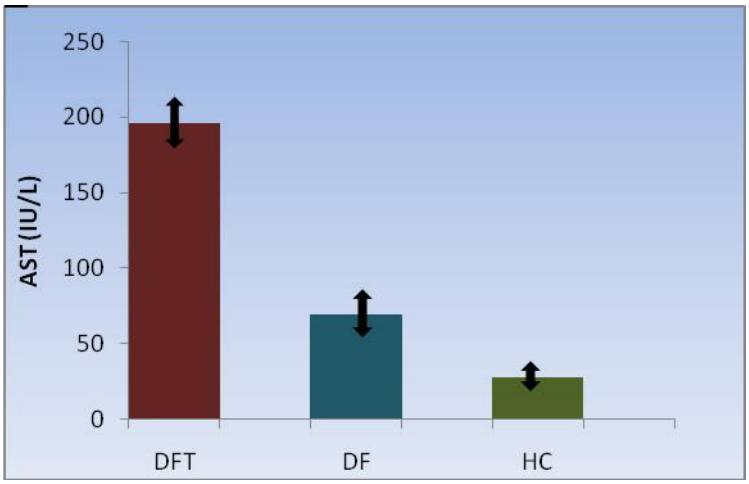
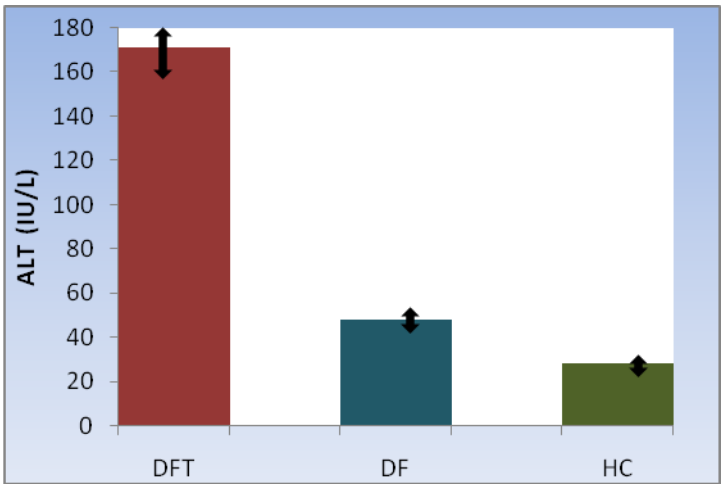


Figure 6: Comparison of ALT level of DFT, DF and HC subjects



Platelet count and AST and ALT level of all the dengue positive cases (DFT and DF) and Human controls were recorded and comparison of counts between the following groups was studied.

Group a: Comparison of platelet count of DFT, DF and HC subjects.

Group b: Comparison of AST level of DFT, DF and HC subjects.

Group c: Comparison of ALT level of DFT, DF and HC subjects as shown in Table 4.

Statistically the differences were found to be significant with p-value <. 0.0001 in group a,b,c respectively. There was a statistical significant difference in mean Platelet count, AST and ALT between DF, FT and HC groups. Mean Platelet count in DFT group was significantly less from DF and HC. Mean AST level was significantly higher among DF group compared to DFT and HC. Mean ALT level was also significantly higher in DF group compared to DFT and HC.

IV. CONCLUSION

The immunochromatographic test should not be used as a standalone device for diagnosing dengue cases. NS1-antigen detection through ICT in combination with MAC-ELISA could help in early and accurate diagnosis of dengue infection. Further population based studies are needed, as dengue has emerged as a significant problem in Karnataka, to suggest an effective diagnostic technique for dengue virus detection.

V. ACKNOWLEDGEMENTS

We are gratefully acknowledging the HOD of Medicine Dr. Sudarshan Murthy and HOD of Pediatrics Dr. Narayanappa for providing us the samples for this work. We are thankful to Assistant Professor of Community Medicine Dr. Praveen Kulkarni for his kind help to carry out the statistical analysis which was required for this study.

REFERENCES

[1] Sumana MN, Linda Rose Jose, Tabasum Begum M. Seroprevalence of Dengue and Leptospira co-infection in Mysore, Karnataka: A study in children at a Tertiary care Hospital. *International Journal of Inventions in Pharmaceutical Sciences, 2(3)*, 2014; 774-778.

[2] World Health Organization, Dengue Hemorrhagic Fever: Diagnosis, treatment, prevention and control. New edition, Geneva, Switzerland: WHO; 2009; 3-144.

[3] Guzman M.G, Kouri, G. Dengue diagnosis, advances and challenges. *International Journal of Infectious Diseases, 8*, 2004, 69-80.

[4] Guidelines for clinical management of Dengue fever, Dengue Hemorrhagic fever and Dengue shock syndrome, Directorate of National Vector Borne Diseases Control Programme, Directorate General of Health Services, Ministry of Health and Family Welfare, New Delhi, 2008; 1-33.

[5] Nivedita Gupta, Sakshi Srivastava, Amita Jain & Umesh C. Chaturvedi. Dengue in India. *Indian Journal of Medical Research, 136*, 2012; 373-390.

[6] Yergolkar P, Hanumayu K, Epidemiological investigations on dengue virus infections in Karnataka and neighboring areas. Annual Report, 2004-2005; 51-67.

[7] Sathish N, Vijayakumar T.S, Abraham P and Sridharan G. Dengue Fever: Its Laboratory Diagnosis with special Emphasis on IgM detection. *Dengue Bulletin, 27*, 2003; 116-125.

[8] Shrivastava A, Dash PK, Tripathi NK, Sahni AK, Gopalan N, Lakshmana Rao PV. Evaluation of a commercial dengue NS1 enzyme-linked immunosorbent assay for early diagnosis of dengue infection. *Indian Journal of Medical Microbiology, 29*, 2011; 51-55.

[9] Kumarasamy V, Wahab AH, Chua SK, Hassan Z, Chem YK, Mohamad M, Chua KB. Evaluation of a commercial dengue NS1 antigen-capture ELISA for laboratory diagnosis of acute dengue virus infection. *Journal of Virological Methods, 140*, 2007; 75-79.

[10] Guidelines for clinical management of dengue fever, dengue hemorrhagic fever and dengue shock syndrome. Directorate of National Vector Borne Disease Control Programme. New Delhi, India: 2008; 11-12.

[11] World Health Organization. Dengue Hemorrhagic fever: Diagnosis, treatment, prevention and control. 2nd edition. Geneva, Switzerland: WHO, 1997; 12-23.

[12] Moorthy M, Chandy S, Selvaraj K, Abraham AM. Evaluation of a rapid immunochromatographic device for the detection of IgM and IgG antibodies to dengue viruses (DENV) in a tertiary care hospital in South India. *Indian Journal of Medical Microbiology, 27*, 2009; 254-256.

[13] Jayasimha VL, Thippeswamy MTR, Yogesh Babu KV, Vinodkumar CS, Niranjan HP, Raghkumar KG, *et al*. Dengue: seroprevalence, comparison of rapid test with ELISA. *National Journal of Basic Medical Sciences, 3*, 2012; 57-60.

[14] Palmer CJ, King SD, Cuadrado RR, Perez E, Baum M, Ager AL. Evaluation of the MRL diagnostics dengue fever virus IgM capture ELISA and the panbio rapid immunochromatographic test for diagnosis of dengue fever in Jamaica. *Journal of Clinical Microbiology, 37*, 1999, 1600-1601.

[15] Pramiladev. R. Kaivalya, Shreeram Kora. Study of rapid serological tests for diagnosis of Dengue. *Scholars Journal of Applied Medical Sciences 1(5)*, 2003, 541-548.

[16] A pilot study on early diagnosis of dengue infection by NS1 Antigen detection and correlation of clinical and laboratory parameters with disease outcome. The Study is Funded by: National Rural Health Mission. Department of Health & Family Welfare, Government of West Bengal, 2012.