Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2016; 4(2D):618-622 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Original Research Article

Evaluation of Correlation between Dengue Serological Markers and Platelet Count

Sindhanai V[1*], Sageera Banoo[2], Rajkumar N[3], Suresh Chander VC[4]
[1]Assistant Professor, [2] Associate Professor, [3]Associate Professor, [4] Professor and HOD, Department of Microbiology, Dhanalakshmi Srinivasan Medical College and Hospital, Perambalur, Tamilnadu, India

***Corresponding author**
Dr. Sindhani V
Email: drsindhanai@gmail.com

Abstract: Dengue is an important public health problem worldwide. Dengue virus infection can produce self-limiting illness to fatal life threatening complications like dengue hemorrhagic fever [DHF] and dengue shock syndrome [DSS]. Dengue specific NS1 antigen, IgM and IgG antibody detection can be used for early diagnosis which is essential for effective management of cases to reduce the mortality and morbidity. The only non-dengue parameter which helps in predicting complications is platelet count. Hence this study was conducted to identify the dengue specific serological markers and to correlate them with platelet count of probable dengue cases. Serum samples were collected from suspected dengue cases and Dengue specific NS1 antigen, IgM antibody and IgG antibody were detected by ELISA method. Platelet counts were obtained for all these cases and correlated with dengue serological markers. Out of 230 samples, 93 were positive for one or more of the three dengue parameters. Among the positive samples, NS1 antigen was detected in 72 cases, antibodies IgM and IgG were detected in 21 cases. 37 cases were exclusively positive for NS1 antigen, 9 and 3 cases were positive alone for IgM and IgG respectively. All three parameters were positive in 24 cases. Thrombocytopenia was seen in 73 dengue seropositive cases. Platelet count is much reduced in secondary dengue infections than the primary infections. Platelet count is a simple but important accessory test which not only supports the diagnosis of dengue and also helps to monitor the disease progression.
Keywords: ELISA, thrombocytopenia, dengue, NS1 antigen, platelet count, dengue serological markers.

INTRODUCTION:

Dengue is an acute febrile illness caused by one of the four serotypes of dengue virus, namely DEN-1, DEN-2, DEN-3 and DEN-4. Dengue virus belongs to flavivirus genus of Family flaviviridae and comes under the group of arboviruses (arthropod borne viruses) [1,2]. Major vector which is transmitting dengue infection in India is *Aedes aegypti* and less commonly by *Aedes albopticus* [3]. According to estimation of World health organization every year there are 50 to 100 million Dengue cases worldwide and 250,000 to 500,000 cases of DHF resulting in 24,000 deaths each year [4]. Dengue and DHF is endemic in more than 100 countries. South East Asia and Western pacific regions are severely affected bearing 75% of global disease burden. South East Asian countries are divided into 3 categories based on endemicity and India has been categorized in Group A where all the 4 serotypes are prevalent [5]. Due to rapid urbanization, lifestyle changes and deficient water management, the risk of dengue infection in India has increased in recent years [6].Dengue virus infection in human beings may result in varied clinical illness ranging from subclinical infection to non-specific febrile illness, classic dengue fever, dengue haemorrhagic fever [DHF- grades I and II], and dengue shock syndrome (DSS - grades III and IV) [7].The combined case fatality rate is around 5% for dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [8]. Hence early, rapid and accurate diagnosis is essential for appropriate clinical management to reduce the mortality rate.

Virus isolation in cell culture, identification of viral genomic sequence by nucleic acid amplification techniques like RT-PCR, NASBA and detection of dengue specific IgM, IgG antibodies are widely used by most of the laboratories for diagnosis of dengue infection [9]. Virus isolation and molecular techniques cannot be used as routine diagnostic tests because they are laborious, time consuming and require specialized laboratory facilities [10]. Serological diagnosis by detection of antibodies is widely used, but antibodies appear only after 4 to 6 days of illness [11]. Secretory protein NS1 antigen is seen in high concentrations during acute phase of illness (1 to 5 days) [12]. Combination of NS1 antigen detection along with

antibody detection increases the diagnostic rates [13]. Immunochromatographic detection of these serological markers yield rapid results but have low sensitivity as compared to ELISA [14].During first 3 days of illness platelet count is normal. Thrombocytopenia begins during febrile phase and platelet count is progressively reduced during hemorrhagic illness [DHF] [15]. As per WHO guidelines, thrombocytopenia can be used as a simple diagnostic criteria for DHF [16].The only accessory laboratory test which supports the diagnosis of dengue is platelet count and it can be roughly estimated by microscopy even in the peripheral laboratories [17].

Hence the present study is designed to correlate the dengue serological markers with platelet count which, not only helps in identifying and categorizing the patient but also in planning management accordingly, thereby curtailing further progression of disease to its severe forms and thus increasing positive prognosis.

MATERIALS AND METHODS:

This retrospective study was carried out for a period of 4 months from September 2015 to December 2015 in a tertiary care hospital. A total of 230 blood samples were collected from clinically suspected cases of dengue fever attending various clinical departments of our hospital. Screening for NS1 antigen, anti-dengue IgM and IgG antibodies by ELISA technique was done.

NS1 antigen testing was done by using Dengue NS1 antigen MICROLISA [direct sandwich principle] kit from J. Mitra& Co. Pvt. Ltd. New Delhi-INDIA. Anti-dengue IgM antibodies were detected by Dengue IgM MICROLISA [MAC CAPTURE ELISA] kit obtained from J. Mitra& Co. Pvt. Ltd. New Delhi-INDIA. Anti-dengue IgG antibodies were detected by Dengue IgG MICROLISA [GAC CAPTURE ELISA] kit obtained from J.Mitra& Co. Pvt. Ltd. New Delhi-INDIA

Manufacturer's instructions were followed strictly while performing the ELISA. Values were calculated and results were interpreted as per manufacturer's guidelines. Platelet counts were obtained for all the screened cases to compare with dengue serological markers. Statistical analysis was done using Quick Calcs online software from GraphPad.

RESULTS:

Out of 230 samples screened in our study, 93[40%] were positive for dengue parameters, 137 [60%] were negative. .

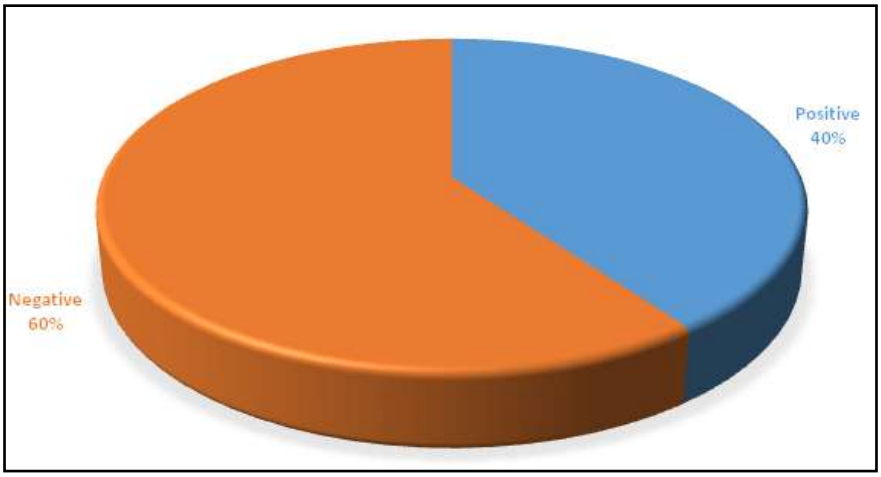

Fig.1: Prevalance of dengue positive cases among screened cases

Table 1: Prevalence of Dengue parameters

Dengue parameters	Total Positive cases	Percentage
NS1 Ag	37	39.7%
IgM Ab	9	9.7%
IgG Ab	3	3.2%
NS1 Ag + IgM Ab	9	9.7%
NS1 Ag + IgG Ab	2	2.2%
IgM Ab + IgG Ab	9	9.7%
NS1 Ag + IgM Ab + IgG Ab	24	25.8%
Total	93	100%

Table 2: Platelet count of probable dengue cases

Platelet count	Dengue positive cases	Dengue negative cases
< 1,00,000/ml	73 [78.5%]	35 [25.5%]
> 1,00,000/ml	20 [21.5%]	102 [74.5%]
Total	**93 [100%]**	**137 [100%]**

Fisher's exact test – p value <0.001, statistically significant

Table 3: Dengue parameters and platelet count comparison

Dengue parameters	**Total positive cases**	**Platelet count** < 1,00,000/ml	**Percentage**
NS1 Ag	37	32	86.5%
IgM Ab	9	4	44.4%
IgG Ab	3	2	66.7%
NS1 Ag + IgM Ab	9	6	66.7%
NS1 Ag + IgG Ab	2	2	100%
IgM Ab + IgG Ab	9	6	66.7%
NS1 Ag + IgM Ab + IgG Ab	24	21	87.5%
Total	**93**	**73**	**78.5%**

Table 4: Comparison of thrombocytopenia in cases with NS1antigen and antibodies:

Dengue positive cases	**Total positive cases**	**No. of cases with thrombocytopenia**	**Percentage [%]**
NS1 antigen positive	72	61	84.7%
Antibodies [IgM & IgG] alone positive	21	12	57.1%
Total	**93**	**73**	**78.5%**

Table 5: Thrombocytopenia in primary vs secondary dengue infections:

Dengue positive cases	**Total number of cases**	**No. of cases with thrombocytopenia**	**Percentage [%]**
Primary dengue infection	55	42	76.4%
Secondary dengue infection	38	31	81.6%

DISCUSSION:

Out of 93 positive cases, 72 [77.04%] cases were positive for NS1antigen either alone or in combination with antibodies. 37 [39.8%] cases were exclusively positive for NS1 antigen only. During first few days of illness NS1 Ag circulates at high level in our blood. The level of NS1 Ag varies from 0.04 - 2 μg/ml in acute-phase serum samples and only 0 .04μg/ml or even less in convalescent phase. Hence NS1 antigen positivity in a person indicates acute phase of illness [8].Dengue specific IgM and IgG antibodies were detected in 22.58% of cases in our study (Ref. Table 1). Dengue specific IgM can be detected in blood only after 3 to 5 days of illness; hence it cannot be used as an early diagnostic marker. IgG antibody is not a reliable marker as it is a cross reacting antibody to other flaviviruses also. When antibodies are used for testing, rise in titre should be demonstrated in acute and convalescent serum, if NS1 antigen is positive no need for repeated testing [18].

Primary and secondary dengue infections produce different immunological response in humans. There will be a low titre and slow rising of antibodies in primary infection and IgM antibody will appear first followed by IgG antibody at the end of first week of illness. In contrast during secondary infection, rapid increase and high titre of antibodies are seen, i.e., high levels of IgG can be detected even during acute phase of secondary infection and IgM response is variable [19]. In our study, primary infection [positive for NS1 Ag, IgM, NS1 + IgM] was seen in 55 [59%] cases and secondary infection [positive for IgG, NS1 + IgG, IgM + IgG, NS1+ IgM+ IgG] was seen in 38 cases [41%]. (Ref. Table 1). Saroj Golia *et al.;* [20] reported 57.4% primary dengue infections and 42.6% secondary dengue infections in their study. Thrombocytopenia in dengue infections is not an early indicator of severe disease but it helps in predicting the progression of disease. On comparison of platelet count with dengue seropositivity, thrombocytopenia [platelet count less than 1 lakh, as per WHO guidelines for DHF] is seen in more number of dengue positive 73[78.5%]cases than dengue negative cases 20 [21.5%] (Ref. Table 2) and the average platelet count of dengue negative cases were higher than the dengue positive cases. Reduction in

platelet count observed in dengue negative cases may be due to other causes like collagen vascular disorders, viral infections other than dengue, drug induced thrombocytopenia etc [21]. In a study conducted by RD Kulkarni et al [22] thrombocytopenia was seen in 68.8% of dengue positive cases and whereas Santosh Shivaji rao Tathe *et al.;* [23] reported 81.72% in their study. On taking the different dengue parameters into account, thrombocytopenia is observed in 61[84.7%] out of 72 NS1 antigen positive cases and 12 [57.1%] out of 21 cases positive of antibodies alone showed thrombocytopenia. Similar findings were observed in the study of RD Kulkarni *et al.;* [22].

Thrombocytopenia was seen in 100% of NS1 + IgG positive cases, 87.5% of cases showing positivity to all three parameters [NS1 + IgM + IgG], 86.5% of only NS 1 antigen positive cases 66.7% of IgG, IgM + IgG, NS1 + IgM, and 44.4% of IgM positive cases (Ref. Table 3). It is clearly observed from the above data that thrombocytopenia was more observed in NS1 + antibodies positive cases than in the NS1 antigen alone positive cases. This signifies the role of antibodies in development of thrombocytopenia. NS1 antigen is detected during first few days of illness during which platelet count is normal and the mechanism of thrombocytopenia in dengue is antibody mediated enhancement. These two factors may be the reason for the above noted observations in our study. RD Kulkarni et al[22] also reported similar observations in their study. This study also showed that thrombocytopenia occurs in secondary dengue cases than the primary dengue cases (Ref. Table 4). Similar findings were observed in the study conducted by Subhash C Arya, Nirmala Agarwal, Satibh C Parikh, and Shekhar Agarwal [24]. Because of smaller sample size, we were not able to statistically correlate the strong association of thrombocytopenia with secondary dengue infections.

CONCLUSION:

Nonspecific symptoms of dengue infection during its early phase necessitate the need for its differentiation from other febrile illness. Platelet count of dengue positive cases were significantly lower than the dengue negative cases. While correlating dengue parameters with platelet count, thrombocytopenia was common in NS1 antigen associated cases than with cases positive for antibodies alone. Thrombocytopenia was much observed in secondary dengue infections than the primary infections. This finding helps the clinician for early prediction and monitoring of the dengue positive case for the development of DHF and DSS thereby reducing the complications.

REFERENCES:

1. Halstead, S. B. Pathogenesis of dengue: challenges to molecular biology. Science 1988; 239: 476–481.
2. Nidhi Pandey, Nagar R, Gupta S; Trend of dengue virus infection at Lucknow, North India (2008- 2010): a hospital based study, Indian J Med Res, 2012; 136(5): 862-867.
3. Pei-Yun Shu, Jyh-Hsiung Huang; Current Advances in Dengue Diagnosis; Clinical and diagnostic laboratory immunology, 2004; 11(4): 642 – 650.
4. WHO; Comprehensive Guidelines for Prevention and Control of Dengue and Dengue Haemorrhagic Fever, Revised and expanded edition, Regional office of SEAR, 2011.
5. Govt. of India; Annual Report 2011-2012, DGHS, Ministry of Health and Family Welfare, New Delhi, 2014.
6. Mairuhu AT, Wagenaar.J, Brandjes DP, van Gorp EC; Dengue: an arthropod-borne disease of global importance. Eur. J. Clin. Microbiol. Infect. Dis., 2004; 23:425-33.
7. Sultana N, Biswas SK, Sultan T, Ahmed S, Hossain Z, Chowdhury R; Seroprevalence of dengue fever in Chittagong, Bangladesh. Chatt Maa Shi Hosp Med Coll J 2013; 12(1): 38–40.
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 J Med Microbiol 2011; 29:51-5.
9. Vijayakumar TS, Sara Chandy, Narayanan Sathish, Mary Abraham, Priya Abraham, Gopalan Sridharan; Is dengue emerging as a major public health problem? Indian J Med Res 2005; 121: 100-107.
10. Santosh ST, Chincholkar VV, Kulkarni DM, Nilekar SL,Ovhal RS, Halgarkar CS; 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
11. Young PR, Hilditch PA, Bletchly C, Halloran W; An antigen capture enzyme-linked immunosorbent assay reveals high levels of the dengue virus protein NS1 in the sera of infected patients. J Clin Microbiol 2000; 38: 1053-7.
12. Anita Chakravarti, Amod Kumar, Sonia Malik; Detection of dengue infection by combining the use of an ns1 antigen based assay with antibody detection. Southeast Asian J Trop Med Public Health 2011; 42(2): 297- 302
13. Fry SR, Meyer M, Semple MG, Simmons CP, Sekaran SD, Huang J.X. *et al.;* The Diagnostic Sensitivity of Dengue Rapid Test Assays Is Significantly Enhanced by Using a Combined Antigen and Antibody Testing Approach, PLOS neglected tropical diseases 2011; 5(6): e1199.

14. Halstead SB, Udomsakdi S, Singharaj P, Nisalak A; Dengue & chikungunya virus infection in man in Thailand, 1962-1964. 3. Clinical, epidemiologic and virologic observations on disease in non-indigenous white persons. Am J Trop Med Hyg 1969; 18: 984-96.
15. Pimpan P, Prasert T; Clinical laboratory investigations. Monograph on dengue/dengue haemorrhagic fever, WHO SEARO; 1993; 62-71.
16. Pruthvi D, Shashikala P, Shenoy V; Evaluation of Platelet Count in Dengue Fever Along with Seasonal Variation of Dengue Infection. J Blood Disorders Transf 2012; 3:128. doi:10.4172/2155-9864.1000128
17. Parameswarappa Jyothi, Basavaraj C; Metri Correlation of serological markers and platelet count in the diagnosis of Dengue virus infection. Adv Biomed Res 2015; 4:26.
18. Peeling RW, Artsob H, Pelegrino JL, Buchy P, Cardosa MJ, Devi S *et al.;* Evaluation of diagnostic tests: Dengue. Nat Rev Microbiol 2010;8: S30-7
19. Philippe Buchy, Sutee Yoksan, Rosanna W; Peeling, Elizabeth Hunsperger; Laboratory Tests For The Diagnosis Of Dengue Virus Infection; Working paper for the Scientific Working Group on Dengue Research, convened by the Special Programme for Research and Training in Tropical Diseases, Geneva, 2006.
20. Saroj Golia, Vivek Hittina halli, K. Sujatha Karjigi, Mallika Reddy, Asha S Kamath; Serodiagnosis of dengue using NS1 antigen, dengue IgM, dengue IgG antibody with correlation of platelet counts. International Journal of AJ Institute of Medical Sciences 2012; 1(2).
21. Peters CJ; Infections caused by arthropod‐ and Rodent ‐born viruses. In: Fauci AS, editor. Harrison's principles of Internal Medicine. 17th ed. New York: McGraw‐Hill Medical Publishing Division; 2008; 1226‐39.
22. RD Kulkarni, SS Patil, GS Ajantha, AK Upadhya, AS Kalabhavi, RM Shubhada, *et al.;* Association of platelet count and serological markers of dengue infection importance of NS1 antigen. Indian Journal of Medical Microbiology, 2011; 29(4): 359-62.
23. Santosh Shivajirao Tathe, Chincholkar VV, Kulkarni DM, Nilekar SL ,Ovhal RS, Halgarkar CS; 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.
24. Subhash C Arya, Nirmala Agarwal, Satibh C Parikh, Shekhar Agarwal; Simultaneous Detection of Dengue NS1 Antigen, IgM plus IgG and Platelet Enumeration during an Outbreak. SQU Med J, 2011; 11(4): 470-476.