Original Article

DOI: 10.21276/APALM.2992

A Study of Correlation Between Dengue Serological Markers and Platelet Count in Ajmer Region

Parmendra Pachori[1], Govind Kumar Sharma[2], Saroj Pachori[1]* and Geeta Pachori[3]

[1]Department of Pathology, SMS Medical College, Jaipur, Rajasthan, India
[2]CHC Bichoon, Jaipur II, Rajasthan India
[3]Department of Pathology, J.L.N. Medical College, Ajmer, Rajasthan, India

ABSTRACT

Background: Dengue is an acute viral infection has emerged as a notable public health problem. Rapid immunochromatographic test and IgM/IgG ELISA has been the mainstay of diagnosis. Combination of NS1 antigen detection along with antibody detection increases the diagnostic rates. Thrombocytopenia begins during febrile phase. Therefore, we tried to evaluate the association of platelet counts against immunochromatographic test (NS1 and IgM/IgG) and IgM ELISA in dengue infections.

Methodology: The study of variation in different platelet parameters in dengue fever cases was undertaken in our department over a period of 18 months from July 2017 to December 2018. Inclusion criteria: All patients with clinical features and serologically positive dengue infection included.

Exclusion Criteria: Patient's which are serologically negative dengue and if routine laboratory test suggesting a bacterial, parasite or any viral infection other than dengue infection or any other disease.

CBC was done from all clinically suspected dengue cases. Serum samples were tested for NS1, IgM and IgG by immunochromatography-based test and IgM MAC-Capture ELISA. Platelet counts were obtained from all serologically positive cases. Test results of dengue specific parameters were compared against platelet counts.

Results: Of 636 samples tested, 396 were positive for one or more dengue parameters. Of the 396, 312 were positive for NS1, 152 were positive for IgM, 41 were positive for IgG and 354 were positive for IgM ELISA. Thrombocytopenia was consistently associated with one of the serological parameters.

Conclusion: It can be concluded from our study that Thrombocytopenia was found in all serological positive dengue cases and showed a significant correlation with serological markers.

Keywords: *Dengue, Serological Markers, Thrombocytopenia, Immunochromatographic Test*

Introduction

Dengue (also called "break bone fever") is a febrile illness caused by a flavivirus transmitted by *Aedes aegypti or Aedes albopictus* mosquitoes during a blood meal. There are five dengue virus (DENV) types (DENV-1, DENV-2, DENV-3, DENV-4 and DENV-5), all of which are capable of inducing severe disease (dengue hemorrhagic fever [DHF]/dengue shock syndrome [DSS]). Dengue is endemic in more than 100 countries in tropical and subtropical regions and causes an estimated 390 million infections annually worldwide, of which 96 million are clinically apparent. [1]

Efficient and accurate diagnosis of dengue is of primary importance for clinical care (i.e., early detection of severe cases, case confirmation and differential diagnosis with other infectious diseases). Laboratory diagnosis methods for confirming dengue virus infection may involve detection of the virus, viral nucleic acid, antigens or antibodies, or a combination of these techniques. After the onset of illness, the virus can be detected in serum, plasma, circulating blood cells and other tissues for 4–5 days. During the early stages of the disease dengue infections may be diagnosed by virus isolation in cell culture, by detection of viral RNA by nucleic acid amplification tests (NAAT), or by detection of viral antigens by ELISA or rapid tests. At the end of the acute phase of infection, serology is the method of choice for diagnosis. [2,3]

Apart from the dengue specific parameters, platelet count is a prognostic laboratory parameter which is available to identify severity of dengue, even though thrombocytopenia is not an early indicator of severe dengue, but it helps in predicting the progression of disease. [4]

Hence the 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.

Materials & Methods

This prospective study of variation in different platelet parameters in dengue fever cases were undertaken at our department over a period of 18 months from July 2017 to December 2018. All patients with clinical features (according to WHO criteria) and serologically positive for dengue infection, presenting to indoor and outdoor departments of our medical College were included in the present study. Laboratory diagnosis of dengue was established by testing of NS1 (Non Structural protein 1), IgM and IgG by immune chromatographic Rapid strip test. Serological confirmation of dengue cases were done by the IgM Enzyme Linked Immuno- sorbent Assay (MAC-ELISA) test in present study.

Results

The table 1 depicts the platelet count of 396 patients at the time of admission, during stay and at the time of discharge. The Mean ± SD at the time of admission was 42.18±18.765 and during the stay was 64.34±20.851 and at the time of discharge was 102.79±27.644. We conclude that at the time of discharge the value was on higher side and improved (Table 1).

In present study there was significant correlation between platelet count and serological markers (NS1 and IgM). There was not any significant correlation between platelet count and IgG. In IgG cases with 20-50,000 and 50-1lac platelet count were positive in 62.88% and 67.57% respectively as compared to < 20,000 platelet count were less positive in 48.21% cases. In ELISA IgM cases were positive in 85.15% in 20-50,000 platelet count and 96.43% were positive in < 20,000 platelet count and 90.09 % cases were positive in 50-1lac platelet count respectively (Table 2).

Discussion

Thrombocytopenia and platelet dysfunction were commonly observed in dengue, both strongly related to the clinical outcome. The WHO guidelines for 2009 reaffirmed that a rapid decline or platelet count below 1,50,000/ cumm of blood are one of the indicators of clinical dengue worsening.

In our study, a low platelet count i.e., thrombocytopenia was observed in dengue positive patients at the time of admission which showed a rise at the time of discharge which indicates improved health of patient after treatment.

Table 1: Platelet Count at the Time of Admission, During Stay and at the Time of Discharge.

	At the time of admission	During stay	At the time of discharge
N	396	396	396
Mean±SD	42.18±18.765	64.34±20.851	102.79±27.644
Minimum	6,000/cumm	11,000/cumm	6,000/cumm
Maximum	98,000/cumm	1,23,000/cumm	2,44,000/cumm

Table 2: Correlation between platelet count and serological markers

Platelet Count (/Cumm)	Total	Strip Test						ELISA	
		NS1Ag		IgM		IgG		IgM	
		Positive	%	Positive	%	Positive	%	Positive	%
<20,000	56	46	82.14	50	89.29	27	48.21	54	96.43
20-50,000	229	173	75.55	185	80.79	144	62.88	195	85.15
50-1lac	111	93	83.78	99	89.19	75	67.57	100	90.09
Total	396	312	78.79	334	84.34	246	62.12	349	89.39

Table 3: Comparison of platelet count in different studies.

Platelet count (/cumm)	Sindhanai et.al. (2016)[13]	Tathe et.al. (2013)[14]	Mehta et.al. (2016)[16]	Kanthikar et.al. (2016)[15]	Our study
< 1,00,000	73 (78.5%)	76 (81.72%)	383 (68 %)	114(84.5%)	338 (85.36%)
>1,00,000	20 (21.5%)	17 (18.28%)	180(31.9%)	21(15.55%)	58 (14.64%)
Total	**93 (100%)**	**93 (100%)**	**563(100%)**	**135(100%)**	**396(100%)**

Table 4: Various dengue parameters in dengue patients as compared to present study .

	No. of patients %					
Dengue parameters	**Ingale SV et al. (2018)[17]**	**Kulkarni et al. (2011)[18]**	**Joshi et al (2018)[19]**	**Jyothi P et al. (2015)[20]**	**Gupta et al. (2019)[21]**	**Our study**
NS1 Ag	84	30	29	62.9	64.3	49.74
IgM Ab	5	50	2	11.3	17	17.17
IgG Ab	0	3	25	4.9	1.5	1.5
NS1 Ag + IgM Ab	10	11	11	9.6	6	19.19
NS1 Ag + IgG Ab	0	0.3	14	1.7	1	1
IgM Ab + IgG Ab	0	6	11	9.6	1	4.79
NS1 Ag + IgM Ab + IgG Ab	1	0	8	0	9	6.56

Many other studies support the present findings that a significant proportion of classic dengue patients also develop thrombocytopenia. Kalayanarooj *et al*., (2002)[5] reported thrombocytopenia in 50.2 % of the DF patients and Shah *et al*., (2006)[6] observed it in 50 % of the patients. Llamas *et al*., (2005)[7] reported thrombocytopenia in 37.7 % of the patients. Itoda *et al*., (2006)[8] observed it in 57 % of patients. On the other hand, low percentages (< 25 %) of the patients with thrombocytopenia were also reported (Pervin *et al*., 2004 [9]; Banerjee *et al*., 2008 [10]; Villar-Centeno *et al*., 2008 [11]). A significant fraction (39 %) of the dengue confirmed patients with thrombocytopenia in this study is thought to be due to direct infection of megakaryocytes by virus leading to increased destruction of the platelets or the presence of antibodies directed against platelets (Lin *et al*., 2001).[12] Peripheral destruction of platelets can occur through the direct interaction of the virus in the platelet, as well as indirectly, since the infection leads to the formation of aggregates platelet-endothelial cells and platelet leucocytes or still to secretion of antiplatelet antibodies and production of factors detrimental to platelets. DENV can induce thrombocytopenia through bone marrow suppression, lysis of megakaryocytes and/or peripheral destruction of platelets.

In present study 338 (85.36%) cases shows platelet count less then 1,00,000/cumm and 58 (14.64%) cases shows platelet count more than 1,00,000/cumm. Similar observations seen in study of Sindhanai *et.al*.,[13] Tathe *et.al*.,[14] & Kanthikar *et.al*.[15]and different results were observed in the study of Mehta *et.al*.[16] In our study we conclude that maximum number of serologically positive patients were seen in cases of thrombocytopenia (Table 3).

Dengue parameters includes NS1 antigen, IgM antibody and IgG antibody. Rapid immunochromatographic strip test and IgM ELISA has been the mainstay of diagnosis. NS1 antigen and IgM antibody test was done to evaluate primary infection and IgG antibody test was done to evaluate secondary infection. Inclusion of NS1 in the diagnosis of dengue increases the early diagnosis (1 to 5 days), so as to avoid complications. Antibodies appear only after 4 to 6 days of illness. Combination of NS1 antigen detection along with antibody detection increases the diagnostic rates. Immunochromatographic test yield rapid results but have low sensitivity as compared to ELISA.

In present study, in Strip test, 197 (49.74%) cases were positive for NS1 Ag followed by 76 (19.19%) cases were positive for NS1 Ag + IgM, 68 (17.17%) cases were positive for the IgM, 26 (6.56%) cases were positive for NS1 Ag + IgM + IgG, 19 (4.79%) cases were positive for IgM + IgG, 6 (1.5%) cases were positive for IgG, , 4 (1%) NS1 Ag + IgG.

These results are comparable with study of Jyothi P *et al* [17], Gupta *et al* [18] ,Ingale SV *et al* [19], Kulkarni *et al* [20] and Joshi *et al* (2018) [21]. (Table 4)

Conclusion

It can be concluded from our study that dengue is the one of the most important mosquito born viral disease in the Ajmer region. On hematological examination all patients with clinical features of dengue showed thrombocytopenia. Serological tests rapid immunochromatographic strip test and IgM MAC-Capture ELISA has been the mainstay of diagnosis. Initially rapid immunochromatographic strip test was done to evaluate primary (by NS1 and IgM) and secondary infection (by IgG). Then gold standard test IgM ELISA was done. Inclusion of NS1 in the diagnosis of dengue increases the early diagnosis (1 to 5 days), so as to avoid complications. Antibodies appears only after 4 to 6 days of illness. Combination of NS1 antigen detection along with antibody detection increases the diagnostic rates. Immunochromatographic test yield rapid results but have low sensitivity as compared to ELISA. The results of this study indicate that thrombocytopenia was more consistently found in serologically dengue positive

patients. Hence the study was 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.

Acknowledgement
Nil

Funding
None

Competing Interests
None declared

References
1. Gubler DJ, Clark GG. Dengue/dengue hemorrhagic fever: the emergence of a global health problem. Emerg Infect Dis. 1995; 1: 55–7.
2. Shu PY & Huang JH. Current advances in dengue diagnosis. Clin Diagn Lab Immunol. 2004; 11:642–50.
3. Ho TS, Wang SM, Lin YS, Liu CC. Clinical and laboratory predictive markers for acute dengue infection. J Biomed Sci. 2013; 20:75.
4. Mourão MP, Lacerda MV, Macedo VO, & Santos JB. Thrombocytopenia in patients with virus infection in the Brazilian Amazon. Platelets. 2007; 18(8):605-12.
5. Kalayanarooj S, Chansiriwongs V and Nimmannitya S. Dengue patients at the Children's Hospital, Bangkok: 1995–1999. Review. Dengue Bulletin. 2002;26: 33–43.
6. Shah GS, Islam S, & Das BK. Clinical and laboratory profile of dengue infection in Children. Kathmandu Univ Med J. 2006; 4(1):40-3.
7. Llamas RR, Ricardo A. Chaín-Castro , Llanes J M, Zepeda MGR, Alvarez TG, et al. Haemorrhagic manifestations in dengue. Rev Biomed. 2005;16: 87-94.
8. Itoda I, Masuda G, Suganuma A, Imamura A, Ajisawa A, Yamada K et al. Clinical features of 62 imported cases of dengue fever in Japan. Am J Trop Med Hyg. 2006;75(3):470–4.
9. Pervin M, Tabassum S, Ali MM, Mamun KZ & Islam MN. Clinical and Laboratory Observations associated with the 2000 Dengue Outbreak in Dhaka, Bangladesh. Dengue Bulletin. 2004;28:96-106.
10. Banerjee M, Chatterjee T, Choudhary GS, Srinivas V, Kataria VK. Dengue: A Clinicohaematological Profile. Med J Armed Forces India. 2008; 64(4):333-336.
11. Villar-Centeno LA, Díaz-Quijano FA, Martínez-Vega RA. Biochemical alterations as markers of dengue hemorrhagic fever. Am J Trop Med Hyg. 2008;78(3):370-4.
12. Lin CF, Lei HY, Liu CC et al. Generation of IgM anti-platelet autoantibody in dengue patients. J Med Virol. 2001. 63(2): 143-9.
13. Sindhanai V, Sageera B, Rajkumar N, & Suresh C, Evaluation of Correlation between Dengue Serological Markers and Platelet Count. *Sch J App Med Sci* 2016;4(2D):618-622.
14. Tathe S, 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.
15. Kanthikar, S. & Kalshetti, V. Correlation of thrombocytopenia and serological markers in early diagnosis of dengue infection with special reference to NS1 antigen. Indian Journal of Pathology and Oncology. 2016. 3. 437-439.
16. Mehta KD, Ghedia B, Sheth S, Khandhediya S, Shingala H, Sinha M. Study of Correlation Between Platelet Count and Serological Markers of Dengue Infection with Importance of NS1 Antigen in Western Region of India National Journal of Laboratory Medicine. 2016, Vol 5(2): 55-59.
17. Jyothi P,& Metri BC Correlation of serological markers and platelet count in the diagnosis of Dengue virus infection. Adv Biomed Res. 2015, 30;4:26.
18. Gupta N., & Bareja R., Diagnosis utility of various serological markers including NSI antigen for timely detection of dengue virus infection. IP International Journal of Medical Microbiology and Tropical Diseases, January-March, 2019;5(1):37-40.
19. Ingale SV, Upadhye AJ, Upadhye JJ. Correlation of serological markers and thrombocytopenia in early diagnosis of dengue infection. Int J Res Med Sci. 2018;6(3):812-816.
20. Kulkarni, RD, Patil SS, Ajantha GS, Upadhya AK, Kalabhavi AS, Shubhada RM 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.
21. Joshi AA, Hematological Parameters in Dengue: The Serological Angle A Study. Int. J. Hematol Res 2018. 4(1): 180-184.

***Corresponding author:**
Dr. Saroj Pachori, Associate Professor, Department of Pathology, SMS Medical College, Jaipur Rajasthan.
Phone: +91 9414391891
Email: govindmbbsaj@gmail.com

Financial or other Competing Interests: None.

Date of Submission : 11/12/2021
Date of Final Revision : 20/12/2021
Date of Acceptance : 28/01/2022
Date of Publication : 28/02/2022