ISSN 2515-8260 Volume 9, Issue 4, Summer 2022

CORRELATION OF SEROLOGICAL MARKERS AND PLATELET COUNT IN DENGUE PATIENTS

1.Dr. Srividya Yeruva ,Assistant professor, Department of Pathology, Osmania medical college, Hyderabad. Telangana, India.
2.Dr. Sowjanya Rakam, Assistant professor, Department of Pathology, Gandhi medical college,/SRRIT&CD,Hyderabad, Telangana, India.
3.Dr.Pravallika Mallipeddi , Senior resident , Department of Pathology, Sir Ronald ross institute of tropical & communicable disease,Hyderabad, Telangana, India.

Corresponding author: Dr. Sowjanya Rakam,

ABSTRACT

Introduction: Dengue virus infection has emerged as a notable public health problem in recent decades . Laboratory evaluation of dengue Serological markers and platelet count help in early diagnosis and can be used as predictor to reduce the morbidity and mortality of dengue disease.

Aim: Aim of this study is to evaluate Dengue serological markers for early diagnosis of cases and to study the correlation between serological markers and platelet count in dengue positive cases .

Materials and Methods: This was a cross-sectional prospective study conducted in the Tertiary health care centre , Hyderabad.. All clinically suspected cases of dengue, reported to various outpatient departments, emergency services and admitted patients in the Hospital were included for this study, from July to September 2018 . The Chi-Square test and statistical software Epi info and MS excel was used to find out the statistical significance of the estimate.

Results: Among 700 blood samples were received from clinically suspected case of dengue , **125 (17.85%)** cases were detected dengue positive out of which **65 (52%)** cases showed platelet count less than 1 lakh. . In confirmed dengue cases, thrombocytopenia is more consistently found in dengue positive cases with only IgM and only NS1 positive cases compared to other serological groups.

Conclusion: Detection of dengue specific serological markers along with platelet count in the diagnosis of dengue, increases the early diagnosis so as to avoid complications significantly and can be used as prognostic marker to reduce the morbidity and moratlity of dengue disease.

Keywords: Dengue, Chi-Square test, thrombocytopenia.

INTRODUCTION

Dengue is the most common disease among all the arthropod borne viral disease [1]**.** Dengue fever is an acute febrile arbo-viral disease affecting the tropical and subtropical regions of the world, which is endemic to Indian subcontinent [2] .The incidence of this disease has increased over the last 50 years with 2.5 billion people living in areas where dengue is endemic .. DHF with mortality in most countries is

5%, primarily among young children and adults . Rapid and sensitive laboratory methods required for early detection of the disease to reduce the morbidity and mortality . More specific methods like virus isolation, genomic RNA detection by PCR, are available but it needs well trained staff and an expensive setup which is not feasible in peripheral hospital settings .

In most cases antigen and antibody (IgG/M) detection by ELISA are commonly used for diagnosis of dengue infection, but time required for appearance of IgM antibody is approximately 4-6 days. Dengue non-structural 1 antigen (NS1) is highly conserved glycoprotein produced in both membrane associated and secretory forms is used as a new biomarker for early diagnosis of dengue infection. NS1 antigen detected by ELISA is present in high concentrations in sera of dengue virus infected patients during early clinical phase of disease. Apart from dengue specific parameters, platelet count is the only laboratory test available and easily performed roughly by microscopy without any expensive setup in the peripheral areas and remote area that can support the diagnosis of DHF or DSS. A drop in platelet count below 1,00,000 per mm^3 is usually found between the third and eighth day of illness so thrombocytopenia and haemo-concentration are used to detect and monitor DHF. Because of limited available resources in healthcare system in the peripheral areas, we tried to correlate the platelet counts and serological tests for NS1 antigen and antibody detection for early diagnosis of dengue fever.

AIMS AND OBJECTIVES

Aim of this study is to evaluate Dengue serological markers for early diagnosis of cases and to study the correlation between serological markers and platelet count in dengue positive cases .

MATERIALS AND METHODS

It was a cross-sectional prospective study conducted in a Tertiary health care centre , Hyderabad.. In this study **700** blood samples were received from clinically suspected case of dengue from **July to September 2018** .All clinically suspected patients experiencing febrile illness consistent with dengue fever with two or more of the following manifestations Headache , Muscle pain , Haemorrhagic manifestation , Retro-orbital pain and Rash were included in study . A specially designed, semi-structured questionnaire form was used to collect the clinical data .

Approximately 5 ml of blood samples were collected from all the clinically suspected cases and the serum was separated and tested for the Dengue NS1 , IgM and IgG by ELISA method , and 2ml of EDTA blood samples were collected and the platelet count was done by automated analyser / microscopy.

NS1 Ag assay

NS1Ag MICROLISA (J. Mitra & Co, New Delhi) test kit was used to perform the test in all 700 samples . NS1Ag MICROLISA is a solid phase enzyme linked immunosorbent assay (ELISA) based on the “Direct Sandwich” principle. Anti-Dengue NS1 antibodies are coated on Microwells with high reactivity for Dengue NS1 antigen. The samples are added in the wells and then enzyme conjugate (monoclonal anti-dengue NS1 antibodies linked to Horseradish peroxidase (HRPO) added. A sandwich complex is formed in the well wherein dengue NS1 (from serum sample) is ‘’trapped’’ or

‘’sandwiched’’ between the antibody and antibody HRPO conjugate. wash buffer is added that will washed off Unbound conjugate. The amount of bound peroxidase is proportional to the concentration of dengue NS1 antigen present in the sample. Upon addition of the substrate buffer and chromogen, a blue colour develops. The intensity of developed blue colour is proportional to the concentration of dengue NS1 antigen in sample. Stop solution is added to limit the enzyme-substrate reaction, and a yellow colour develops which is finally read at 450 nm spectrophotometrically. Sample results were expressed in terms of ratio within 2 hours. As per the manufacturer’s guideline, interpretation was done as (i) non reactive for dengue virus NS1 Ag if ratio < 9, (ii) equivocal for dengue virus NS1 Ag if between 9 to 11, and (iii) reactive for dengue virus NS1 Ag if > 11 or more was obtained.

Detection of IgM and IgG antibodies

All the 700 samples were tested for the presence of dengue specific IgM and IgG antibodies by using ELISA kits , developed and commercialized by NIV (National Institute of Virology), Pune, and recommended by National Vector Borne Disease control programme. Tests were done and results were read as per the literature provided.

Platelet Count

EDTA blood samples were collected for platelet count analysis . Platelet count was done by automated analyser and cross checked by light microscopy, which were interpreted as normal, when the count was between 1500000-450000/ cmm and **DHF when the count was < 1,00, 000/ cmm (WHO cut off for platelet count for DHF).**

- The Chi-Square test and statistical software Epi info and MS excel was used to find out the statistical significance of the estimate.

RESULTS

Among 700 blood samples received from clinically suspected case of dengue , 125 (17.85%) cases were detected dengue positive

DEMOGRAPHIC DETAILS OF 125 POSITIVE PATIENT SAMPLES

Age group	**No.of Dengue positive cases (n = 125)**	**% positivity**
0-20 yrs	30	24%
20-40 yrs	55	44%
40-60 yrs	25	20%
>60 yrs	15	12%

- Among all 125 Dengue positive patients , Males were 77 (61.6 %) and Females were 48 (38.4 %)

- Male preponderance is seen in our study with Male : Female ratio 1: 1.6

SEROLOGICAL GROUP CLASSIFICATION OF DENGUE CASES

SEROLOGICAL GROUPS	NO. Positive cases (n= 125)	PERCENTAGE(%)
NS1 positive	25	20%
IgM positive	45	36%
IgG positive	10	08%
NS1 + IgM positive	15	12%
IgM + IgG positive	22	17.6%
NS1 + IgM + IgG positive	08	6.4%

Maximum 45 (36%) number cases present at Mid phase of active infection in our study

THROMBOCYTOPENIA IN DENGUE POSITIVE CASES

PLATELET COUNT	DENGUE POSITIVE CASES	PERCENTAGE
< 1LAKH	65	52%
>1LAKH	60	48%
TOTAL	**125**	**100%**

Maximum 65 (52%) number of dengue positive cases showed thrombocytopenia in our study.

COMPARISION OF DENGUE SPECIFIC SEROLOGICAL PARAMETERS AND PLATELET COUNT

Dengue parameters	**No. of dengue positive patients**	**Percentage (n=125)**	**No. of cases with Platelet count < 1 lakh**	**Percentage (n=65)**
Only NS1 Ag positive	25	20%	18	27.69%
Only IgM positive	45	36%	22	33.84%
Only IgG positive	10	8%	04	6.15%
NS1+IgM positive	15	12%	11	16.92%
NS1 +IgG positive	0	0%	0	0%
IgM+IgG positive	22	17.6%	06	9.23%
NS1+IgM+IgG positive	8	6.4%	04	6.15%
	125		**65**	

In our study , Among Dengue positive cases 65 (52%) cases showed platelet count less than 1 lakh. Platelet count decreased more in IgM positive group (33.84%) followed by only NS1Ag positive cases (27.69%), than in other groups. Platelet count is almost normal in most cases ,when IgG antibody is positive.

DENGUE SEROLOGICAL MARKERS INTERPRETATION CHART

S.NO	**NS1 Ag**	**IgM(Ab)**	**IgG(Ab)**	**Interpretation**
1.	+	-	-	Early infection
2.	+	+	-	Acute phase of infection
3.	-	+	-	Mid phase of active infection (early recovery)

4.	-	+	+	Late phase of active infection
5.	-	-	+	Past infection / patient recovered
6.	+	-/+	+	Secondary infection / current active infection
7.	-	-	-	No infection

DISCUSSION

Dengue is the most common arthropod borne viral diseases. Dengue infection was generally encountered in India during or after rains, due to rise in vector population. The raise in dengue cases is normally seen during July or August and it may continue till September or October [1]. A total of 700 serum samples were received to our laboratory from clinically suspected cases of dengue from July to September 2018.Among them 125(17.85%) samples were dengue positive by either one or more of three markers tested [NS1, IgM, IgG] which is in correlation with Parameshwarappa Jyothi et al (11.92 %) [2] , Manoj kumar et al (23%) [3] studies. In contrast higher positivity rate was seen in Suchita vikas ingale et al.. study (50%) [4].

Male preponderance was observed in our study with male to female ratio of 1.6 :1 . Similar results were seen in studies conducted by Manoj kumar et al. (1.54 :1) [3], Suchita vikas ingale et al. (1.2 :1) [4] studies, In contrast Chakravarthi A et al. [5] study greater propensity in female gender was seen. The studies from several countries in Asia have shown a consistent pattern of males being more commonly affected. It might be due to differences in sociocultural environment where male are more exposed to outdoor activities and their bodies less covered as compared to that of females [2]. In our study Predominant age group affected by dengue infection was 20 – 40years with positivity of 55 cases (44%) ,which is in contrast to many studies Shah I. et al. [6], Hoti et al [7] where in children are most commonly effected but findings similar to our study were seen in Suchita vikas ingale et al. [4] study wherein majority positive (85%) cases were found in patients above 15years age.

Dengue PCR is useful in early dengue infection, but it is costly and not available everywhere. NS 1 antigen is used in the early phase of dengue infection. Hence identifying IgM, IgG and NS1 specific to virus remains as important diagnostic parameters. In our study , NS1 antigen, anti-dengue IgM and IgG antibodies were tested by ELISA technique. Out of total 125 positive cases 25 (20%) were positive for NS1 antigen and 15 cases (12%) positive in combination with IgM. 45 cases (36%) were exclusively

IgM positive. Overall in our study primary cases (positive for NS1, IgM, NS1+IgM) was seen in 85 (68%) cases and secondary infections (Positive for IgG, NS1+IgG ,IgM+ IgG , NS1+IgM +IgG) was seen in 40 (32%) cases. It is in correspondence with Golia S et al.study wherein 57.4% cases of dengue were primary infections and 42.6% cases were secondary dengue infections [8] . In our study Maximum number of cases 45 (36%) are present at mid phase of active infection (IgM positive) which is correlation with SN kanthikar et al study [9] in which out of 135 dengue positive cases, majority 57(63.33%) cases were positive for IgM. Among two antibodies, IgG is a less reliable marker in the diagnosis of DI. Both clinical and sub-clinical infections can produce IgG which may persist for several years affecting the interpretation of test results.

This study also tried to find the association of dengue parameter with thrombocytopenia. Of the 125 total dengue positive cases, 52 (65 %) cases showed thrombocytopenia, when IgM antibodies were considered for the diagnosis of DI, thrombocytopenia was noted in 22 (33.84%) cases. In our study the association of thrombocytopenia with IgM was found to be higher in contrast to studies of R.D. Kulkarni et al,[10] in which association of thrombocytopenia with NS1 Ag was found to be higher that is (71.2%).

Higher association of IgM and thrombocytopenia in our study may be due to most of the cases coming to our hospital were referred from various places. This may result in slight delay in diagnosis and thus by that time acute antibody production will be started in the body and also patient condition may deteriorates by decrease platelet count and increasing morbidity. 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 very useful in early and accurate diagnosis of dengue.[4]

CONCLUSION

Detection of NS1Ag in the diagnosis of dengue increases the early diagnosis so as to avoid complications significantly. In cases of fever, thrombocytopenia is more consistently found in dengue positive subjects with only IgM and only NS1 positive cases than in other serological groups and can be used as an early indicator to reduce the complication of dengue disease. Correlation of platelet count with serological marker helps in early diagnosis as well as prognostic marker in larger study groups.

ACKNOWLEDGMENT

The author is thankful to Department of Pathology for providing all the facilities to carry out this work.

Conflict of Interest

None

Funding Support

Nil

REFERENCES

1.S.L. Annapoorna, M.L. Kavitha Latha, Shanker. Clinical, microbiological profile of dengue cases reported at a tertiary care hospital in Telangana, India. Trop J Path Micro 2019;5(11):899-902.doi:10.17511/jopm.2019.i11.11.

2. Jyothi P, Metri BC. Correlation of serological markers and platelet count in the diagnosis of Dengue virus infection. Adv Biomed Res 2015;4:26.

3. Kumar M, Verma RK, Mishra B. The prevalence of dengue fever in Western Uttar Pradesh, India: A gender-based study. Int J App Basic Med Res 2020;10:8-11.

4. Ingale SV, Upadhye AJ, Upadhye JJ. Correlation of serological markers and thrombocytopenia in early diagnosis of dengue infection. Int J Res Med Sci 2018;6:812-6.

5.Chakravarti A, Arora R, Luxemburger C. Fifty years of dengue in India. Trans R Soc Trop Med Hyg 2012;106:273-82.

6.Shah I, Katira B. Clinical and Laboratory Abnormalities due to Dengue in Hospitalized children in Mumbai in 2004. Dengue Bull. 2005;29;95-96.

7. Hoti S L, Soundravally R, Rajendran G, Das L K, Ravi R, Das P K. Dengue and dengue hemorrhagic fever outbreak in Pondicherry, South India, during 2003-2004, Emergence of DENV -3. Dengue Bull. 2006;30:42-50.

8 .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. Inter J AJ Institute Med Sci. 2012;1(2).

9. Kanthikar SN, Kalshetti VT. Correlation of thrombocytopenia and serological markers in early diagnosis of dengue infection with special reference to NS1 antigen. Ind J Pathol Oncol. 2016;3(3):437-9.

10.Kulkarni RD, Patil SS, Ajantha GS, Upadhya AK, Kalabhavi AS, [11]Subhada RM et al. Association of platelet count and serological markers of dengue infection – importance of NS1 antigen. *Indian Journal of Medical Microbiology*. 2019; 29(4): 359-62.