



ORIGINAL ARTICLE

Correlation of serological markers and thrombocytopenia in Dengue infection - a cross sectional study from 2019 epidemic in Rawalpindi, Pakistan.

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ABSTRACT... Objective: To recognize the spectrum of serological markers found in dengue cases and to associate them with the platelet count. **Study Design:** Cross Sectional Study. **Setting:** Department of Medicine, District Head Quarter Hospital, and Rawalpindi, Pakistan. **Period:** August to November 2019. **Material & Methods:** Serum was analyzed to detect markers of NS1 antigen, IgM and IgG using the ELSIA technique. The platelet count was likewise noted in all of the samples. Data was analyzed by SPSS v25.0. **Results:** 226 patients with dengue fever were enrolled in the study samples. Among 226 examples 63(27.9%) were positive for isolated NS1 antigen and 10 (4.4%) were positive for isolated IgM. Out of 134 positive primary infection cases, 108(80.5%) had thrombocytopenia while out of 92 secondary infection cases, 74(80.4%) had thrombocyte count <100,000/mm³. Overall, thrombocytopenia was recorded in 182(80.5%) out of 226 dengue positive cases. **Conclusion:** In the present research work, the relationship of low platelet count in dengue-parameters positive cases was seen as exceptionally critical. The thrombocyte count, in addition to dengue-specific parameters, is the main available diagnostic test in less developed areas that may be used to confirm the diagnosis of dengue infection.

Key words: Dengue Serological Marker, NS1 antigen, Platelet Count, Thrombocytopenia.

INTRODUCTION

Infection of dengue is one of the most prevalent viral diseases transmitted by mosquitoes in the world, with potentially fatal complications.¹ It is caused by four different serotypes of Flavivirus, all of which are members of the genus Flavivirus and Flaviviridae family. *Aedes aegypti* mosquitoes are the known vector to transmit this virus particularly.²⁻⁴ Globally, this systemic viral disease is responsible for 58.4 million cases, prompting a gauge of 10 000–20 000 deaths every year.² There were around 96 million clear dengue cases all around the world in 2010. Asia shared 70% of this weight. It is more common in thickly populated area and hence India alone contributed 34%. On the other hand Americans contributed 14% of this disease around the world, of which over half happened in Brazil and Mexico.⁵ One serotype infection gives lifetime immunity to this one serotype, while other serotypes lead to the cross-reactivity. Because of

this cross reactivity, the infection may progress to the more complicated end, resulting in Dengue shock syndrome and Dengue hemorrhagic fever, among other complications.^{6,7} Following this brief cross-protection period, quantities of prior heterologous antibodies, which arise from primary infection, can promote viral cell absorption and enhance replication of viruses, leading to more serious symptoms during secondary infection. After secondary infection, individuals are usually assured of all serotypes because postsecondary infections have been observed as hospitalized cases sometimes.^{8,9}

Thrombocytopenia in individuals with either moderate or severe instances of dengue infection is a common clinical symptom. Research suggests that decreased platelet counts in these patients are one of the major causes of hemorrhage. The platelet normal range

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is from 150,000–450,000 platelets/ μ l and in many individuals it may reach <40000 thrombocytes/ μ l from the third to seventh day of fever.¹⁰ Patients may need platelet infusions in order to maintain their haemostatic function at normal physiological levels in certain situations.^{11,12} Platelets activation and malfunctioning are concerned in the prothrombotic consequences of Dengue shock syndrome and Dengue hemorrhagic fever, according to the reported studies by many researchers.^{13,14}

The patient screening for IgM and IgG antibody has been the backbone for diagnosis of Dengue infection. Antibody detection is an alternate strategy for diagnosis and, accordingly, is susceptible to false positive just as false negative outcomes.¹ The most recent criterion NS1 is exceptionally specific and sensitive for the analysis of dengue disease from the absolute first day of fever while platelet count is the main complimentary diagnostic.¹⁵ Platelet count can be generally evaluated by microscopic technique. Aside from the dengue-specific antigen and antibodies, platelet count is one of the significant predictive markers to help in early diagnosis.¹⁶ In less resourceful centers; platelet counts are only available supplementary tests to assist Dengue shock syndrome and Dengue hemorrhagic fever diagnosis. A rough estimation of platelet count by microscopy in resource-limited settings of primary care is helpful in diagnosis and monitoring the treatment of infection.

Duyen et al. have indicated that higher NS1 antigen levels on day 3 of disease were related with lower platelet count.¹⁷ In the current study, we have correlated the platelet counts, and dengue parameters detected by ELISA based dengue serology tests which will help the clinicians in primary care setup to diagnose and monitor the treatment of dengue virus.

MATERIAL & METHODS

This retrospective cross-sectional study was piloted for duration of 3 months from August 2019 to November 2019 at DHQ hospital, Rawalpindi, during the dengue outbreak which included a

total of 226 consecutive samples of blood taken from patients suspected for dengue infection. The criteria for inclusion in our study were all the patients of both the gender with clinical characteristics indicating Dengue infection, confirmed by serological marker while the criteria for exclusion was other identified specific infections, concomitant illnesses, bleeding diathesis and immune-compromised patients. From all the included patents in our study, informed consent was taken. Study approval was given by the Institutional Review Board of Rawalpindi Medical University.

All the serum samples were analyzed to detect markers of NS1 antigen, IgM and IgG using the ELSIA technique while Total leucocyte count and Platelet count were noted. Primary infection was defined as the presence of NS1 antigen alone, IgM antigen alone or the presence of both NS1 and IgM. On the other hand, for a sample to be labeled as a secondary infection, following dengue parameters had to be positive: 1) IgG only 2) NS1 and IgG 3) IgM and IgG 4) NS1, IgG and IgM. Samples were categorized into two groups based on TLC ($>4000/\text{mm}^3$ and $<4000/\text{mm}^3$) and platelet count ($>100,000/\text{mm}^3$ and $<100,000/\text{mm}^3$) results of dengue specific parameters and the type of infection (primary and secondary) was compared against platelet count. Statistical analysis was performed via SPSSv.25.0 software. Descriptive data was represented as frequencies in percentage.

RESULTS

A total of 226 samples were included in our study. Out of 226 samples, 174 (77%) were males while 52 (23%) were females. 139 (61.5%) patients were under 35 years old while 87 (38.5%) were over 15 years old. (Table-I) As can be seen from the table under the heading of Serology markers, out of 226 positive cases, 196 (86.7%) cases were NS1 antigen positive; each alone or combined with antibodies. A total of 63 patients (27.9 %) were solely tested positive for the NS1 antigen. Primary infection in our study was seen in 134 (59.3%) cases and secondary infection was observed in 92 (40.7%) cases. (Table-I)

Variable	N=226
Gender	
Male	174(77%)
Female	52 (23%)
Age	
<35 years	139 (61.5%)
≥35 years	87 (38.5%)
Serology Markers	
NS1 Ag	63 (27.9%)
IgM Ab	10 (4.4%)
IgG Ab	6 (2.7%)
NS1 Ag + IgM Ab	55 (24.3%)
NS1 Ag + IgG Ab	33 (14.6%)
IgM Ab + IgG Ab	14 (6.2%)
NS1 Ag + IgM Ab + IgG Ab	45 (19.9%)
Type of infection based on serological markers	
PRIMARY	134 (59.3%)
SECONDARY	92 (40.7%)
Blood Parameters	
TLC (cells/mm³)	
≥4000cells/mm ³	77 (34.1%)
<4000cells/mm ³	148 (65.5%)
Platelet count (cells/mm³)	
≥100,000cells/mm ³	44 (19.5%)
<100,000cells/mm ³	182 (80.5%)

Table-I. Descriptive statistics for DHF (dengue hemorrhagic fever) patients. (n=226)

In our investigation, out of 226 positive cases, leucopenia was seen in 148 (65.5%) of patients while 77 (34.1%) had normal total leucocyte count. Out of 226 positive cases, thrombocytopenia of <100,000/ml was observed in 182(80.5%) of patients while 44 (19.5%) had platelet count of >100,000/ml.

Moreover, Table-II illustrates the comparison of platelet count with dengue seropositivity. In a sum of 226 cases, thrombocytopenia was seen in 182 cases (80.5%). Thrombocytopenia was observed in 157 (80.10 %) of the 196 cases that were tested positive for NS1; however, when the antibodies alone were examined, thrombocytopenia was observed in 25 (83.33 %) cases out of total 30 cases. Thrombocytopenia was detected in 48 (76.19 %) cases of the 63 cases that were alone positive for

NS1, whereas thrombocytopenia was detected in 46 (83.6%) cases out of 55 cases when NS1 and IgM antibodies were evaluated.

Figure-1 demonstrates that out of 134 cases of primary infection, less than 100,000 platelets were seen in 108 patients (80.59%). 92 cases of secondary infection were reported, out of which only 72 patients had thrombocytopenia (<100,000/ml).

Dengue Parameters	Total Positive Cases	Platelet Count <1,00,000 cells/mm ³ (%)
NS1 Ag	63	48 (76.19%)
IgM Ab	10	10 (100%)
IgG Ab	6	5 (83.3%)
NS1 Ag + IgM Ab	55	46 (83.63%)
NS1 Ag + IgG Ab	33	27 (81.81%)
IgM Ab + IgG Ab	14	10 (71.42%)
Ns1 Ag + IgM Ab + IgG Ab	45	36 (80%)
Total	226	182 (80.53%)

Table-II. Dengue parameters and platelet count comparison.

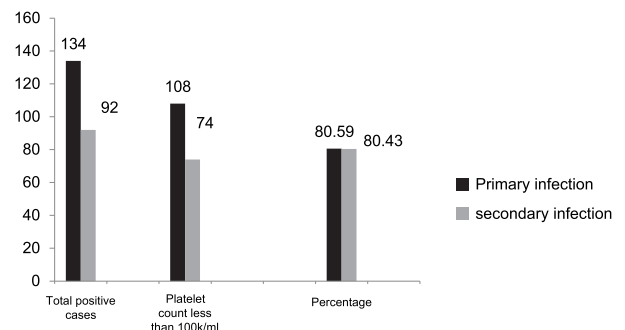


Figure-1. Infection based on serological markers and platelet count comparison.

DISCUSSION

In our study, 139 (61.5%) cases were less than 35 years of age while 87 (38.5%) were above 35 years of age. In a previous study, amongst 1466 patients of dengue, the youngest dengue patient was 8 months old, while the oldest patient was 89 years old, according to a research performed in Nigeria Sembilan in 2010. The average age in their study was 32.2 years old.¹⁸ Out of 226 samples,

174 (77%) were males while 52 (23%) were females in our research work. A study conducted by Kulkarni SK reported that there were 95 male (63.33%) and 55 females (36.66%), with 1.73:1 male to female ratio.¹⁹ A previous study done by Mohan Kashinkunti found that male were 54% and females were 46% in their study.²⁰

In our study, out of 226 positive cases, 196 (86.7%) cases were NS1 antigen positive; each alone or combined with antibodies. A total of 63 patients (27.9%) were solely tested positive for the NS1 antigen. During first few days of illness, NS1 Ag circulates at high level in blood. Thus, positive NS1 antigen test in a patient shows acute phase of illness. Dengue specific IgM can be detected in blood only after 3 to 5 days of disease; hence it cannot be utilized as an early analytic marker.

SN Kanthikar et al found that out of suspected 358 serum samples tested at central clinical laboratory, 135 cases were NS1 marker, IgM or IgG antibody positive. Majority of 57 (63.33%) cases were IgM positive, followed by NS1 marker and IgG antibody with 45 (33.33%) and 7 (5.1%) cases respectively.²¹

Primary infection in our study was seen in 134 (59.3%) cases and secondary infection was observed in 92 (40.7%) cases. Comparable results to our findings were observed by Saroj Golia et al who reported 57.4% primary dengue infections and 42.6% secondary dengue infections in their study.²²

Leukopenia was noticed in 148 (65.5%) of patients while 77 (34.1%) had total leukocyte count within normal range in this study. Leukocytosis was observed by previous study in patients with the CD in the first days of the disease, followed by leucopenia. Leucopenia was more noticeable in the under 15-year-old age group.²³ In our study, out of 226 positive cases, thrombocytopenia of less than 1 lakh was observed in 182 (80.5%) of patients while 44 (19.5%) had platelet count of more than 1 lakh.

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 182 (80.5%) cases.

In our study, out of 134 cases of primary infection, 108(80.59%) patients had platelet count less than 100,000. Moreover, 92 secondary infection cases were reported, out of which only 74 (80.43%) had platelets less than 100,000. As a whole, comparison of infection on the basis of serological markers and platelet count showed that out of 226 cases, 182 patients had thrombocytopenia.

Kotresh Doddamane et al reported that platelet count at admission times was below 10000 in 7.5% of the patients and above 100000 in 22.5% of the patients. He observed that 45 % of the patients tested positive for IgM anti-dengue antibodies, and 55% cases were tested positive for both IgM and IgG antibodies against dengue.²⁴

Because the NS1 marker is the first to be detected in blood from the first day of fever is a useful diagnostic tool for early detection to prevent complication caused by dengue infection. Platelet count has been the only measure of laboratory that may assist dengue infection diagnosis that is done in periphery areas since it is costs efficient and simple to execute without needing an expensive laboratory setup. These estimations will aid in reducing complications caused by late treatment and initiating preventative and control measures far in advance of the development of the disease. As a result, research like these will have a major impact on clinical treatment and may help to decrease morbidity and death associated with dengue infection. The major limitation of our study was small sample size.

CONCLUSION

We concluded in our study that thrombocytopenia is a not an indicator of severity of disease neither related to an indicator of complications like DHF; however thrombocytopenia is a marker for progression of disease, our study confirmed NS1 to be highly sensitive from day one for diagnosis

of dengue fever; however IgM cannot be an early diagnostic marker for dengue fever.






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2	Maha Batool	Experimental / study conduction, Manuscript writing.	
3	Tahira Yasmeen	Experimental / study conduction, Facilitation and Material analysis.	
4	Shehzad Manzoor	Conception of Study, Critical review, Facilitation and Material analysis.	
5	Hamza Waqar Bhatti	Analysis / Interpretation / Discussion Manuscript writing.	
6	Shumaila Mumtaz	Analysis / Interpretation / Discussion Manuscript writing.	