Original Article

Positive Predictive Value of Thrombocytopenia and Leukopenia in Diagnosis of Acute Dengue Infection

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Abstract

Objective: To determine the positive predictive value of thrombocytopenia and leukopenia in patients of acute dengue infection.

Methods: This descriptive study was undertaken in Haematology Department, Medical and Pediatric wards, Fauji Foundation Hospital, Rawalpindi. A total of 100 patients diagnosed as a case of acute dengue infection on the basis of Indirect ELISA for anti DENV IgM were selected using inclusion and exclusion criteria. Venous blood of 2.5 ml of these patients was collected in CP bottle containing EDTA as anticoagulant. It was analyzed on automated Haematology analyzer Sysmex XT 2000i system. Blood levels of Total Leucocyte Count and platelet count were recorded and documented for these patients and their positive predictive values were calculated.

Results: Out of 100 diagnosed patients of acute dengue infection, 81% exhibited thrombocytopenia and 75% had leukopenia. The combined PPV of leukopenia and thrombocytopenia was 88%.

Conclusion: Acute dengue fever is characterized by thrombocytopenia and leukopenia. Therefore Dengue should be the top most differential diagnosis in febrile patients with thrombocytopenia and leucopenia.

Key words: Acute dengue infection, Positive predictive value (PPV), Leukopenia, Thrombocytopenia.

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Introduction

Dengue fever is amongst the most imperative rapidly spreading viral ailment occurs by the bite of mosquito, being most pervasive in sub-tropical and tropical areas.[1,2] Aedes aegypt, a day biting mosquito transmits the virus and disease is characterized by an acute swift occurrence of fever for 3-5 days, myalgia, intense headache, retro-orbital pain, joint pain, gastrointestinal disturbance, anorexia and rash.[1,3]

At present, above 125 countries consisting Pakistan are dengue endemic. Multiple dengue outbreaks have been reported from Pakistan throughout the year peaking at post-monsoon period. Contributing factors in wide spread of dengue fever consisted of genomic evolution in virus, climate change and social influences like elevated urbanization, higher growth of population, socio-economic factors and global trade and travel.[4]

More than 70% global burden of dengue fever is carried by Asian countries and posed as major cause morbidity and mortality among children. Owing to this alarming situation, World Health Organization (WHO) has incorporated it amongst the most important neglected tropical diseases through 2015-2020.[5,6]

Other than dengue, diseases that present as acute febrile illness includes leptospirosis, malaria, Salmonella typhi, Influenza A, Rickettsia, chikungunya and Japanese encephalitis. Since their clinical presentation is similar and overlapping, laboratory confirmation is essential for an accurate diagnosis.[5,7,8]

Correct diagnosis of the pathogen is utmost importance since different management options are required for each.[8] The lab tests that are commonly applied for diagnosis are detection of virus by PCR, detection of viral products like NS1 protein detection, detection of host immune response to infection through IgM and IgG serology. The timing of the appearance and duration of these biomarkers in both primary and secondary dengue infection is variable.[8]

Given the varying kinetics of each biomarker, no single assay can be used to definitively diagnose dengue

patients who may present at a different stage in their infection. NS1 antigen is considered the most robust of all the diagnostic methods. This test may also be obscured by immune complex formation in secondary infections, thereby shortening its window of detection. In this case, combining NS1 detection with IgM and/or IgG detection has been shown to dramatically improve positive dengue diagnosis.[8]

Nevertheless, these serological tests may be inaccessible or cost ineffective in underdeveloped countries or in some small local hospitals, so the clinical clues from the history taking, physical examination and the routine laboratory tests are still important. There was a study in Puerto Rico in 2011 which revealed that the dengue patients had enhanced leukopenia at 87%. Hence patients with acute febrile illness with leukopenia were more likely to be infected with dengue than influenza or other febrile illnesses.[9] Similarly CBC also showed thrombocytopenia of varying degree in dengue fever.

Since CBC is readily available tests in Pakistan even in remote local hospital setting our aim was to identify a positive predictive value of leukopenia and thrombocytopenia in diagnosis of dengue infection. This could help isolating the dengue infection from the other acute febrile illness and further narrow down the number of patients needing a referral to a central lab.

Methods

It was a descriptive cross sectional study. The study was carried after taking permission from ethical committee at Haematology Department Fauji Foundation Hospital, in collaboration with Serology Department Fauji Foundation Hospital. The span of this study was from October 2016 to March 2017.

A total of 100 patients were included in the study. Adults and children of both gender, admitted in pediatric and medical wards having acute dengue infection were enrolled. The dengue infection was confirmed by serological test using an Indirect ELISA for anti DENV IgM (Dengue Virus IgM). Patients who presented with signs of shock, having chronic illness at presentation or having an initial non dengue diagnosis were excluded from the study. An informed consent was taken before enrollment and sampling of the patient. Consecutive non-probability sampling was applied in the study. Charges of the lab test were borne by hospital administration and not the patient.

Venous blood of 2.5 ml of the selected patient group was collected in CP bottle containing EDTA (ethylene diamine tetra acetic acid) as anticoagulant. It was analyzed on automated Haematology analyzer Sysmex XT 2000i system. Total leucocyte count(TLC) and platelet count were noted. A proforma was designed in which blood levels of TLC and platelet count were recorded.

All data was analyzed using software SPSS version 17. Descriptive statistics were used to calculate qualitative and quantitative variables. Qualitative variables i.e. gender, presence of thrombocytopenia and leucopenia were measured as frequency and percentages and quantitative variable i.e. age was measured as mean and standard deviation. Positive predictive value was calculated for individual parameters as well as their combination.

Results

A total of 100 dengue positive patients were enrolled in the study diagnosed on the basis of IgM ELISA. Collectively the age of the patients ranged from 5 to 50 years. Patients were divided into five groups according to each decade of age. Mean age of the patients was 26.4 years. Summary of the age statistics is shown in table I.

Out of 100 patients 50 were males, and 50 were females. Overall 81 out of 100 patients had thrombocytopenia while 19 patients had normal platelet counts .75 out of 100 patients had leucopenia.

The frequency of thrombocytopenia in male versus

Table 1: *Mean and Standard Deviation of Age of Dengue Patients*

	Age Descriptive Statistics				
S	**No. of Patients**	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Age	100	5	50	26.40	14.261

Table 2: *Frequency of Thrombocytopenia Relative to Age Group*

	Male			**Female**		
Age	**No**	**Yes**	**Total Patients**	**No**	**Yes**	**Total Patients**
5-10	0	3	3	0	3	3
>10-20	7	23	30	3	12	15
> 20-30	3	5	8	1	4	5
> 30-40	0	4	4	1	7	8
> 40-50	2	3	5	2	17	19
Total	**12**	**38**	**50**	**7**	**43**	**50**

Thrombocytopenia: platelet count < 150,000/microlitre.

female relative to age group is shown in table II. Thrombocytopenia was most frequently seen in the age group of >10 to 20 years in both genders, while an additional peak was found in females in age group > 40-50 years also shown in table II.

Out of 81 patients with thrombocytopenia, 66 patients had leucopenia. The incidence of thrombocytopenia relative to TLC is shown in table III. This relationship is also verified by the correlation matrix given in table IV.

The positive predictive value (PPV) of thrombocytopenia was 81%, while that of leucopenia was found to be 75% in patients of acute dengue infection. The PPV for the combination of leucopenia and thrombocytopenia was 88%.

Table 3: *Incidence of Thrombocytopenia Relative to TLC*

	Thrombocytopenia		
TLC	**No**	**Yes**	**Total**
Leucopenia present	9	66	75
Leucopenia absent	10	15	25
Total	**19**	**81**	**100**

TLC: total leucocyte count, Leucopenia : TLC < 4000/microlitre

Table 4: *Correlation Matrix*

	TLC	**Thrombocytopenia**
TLC	1	-.390**
Thrombocytopenia	-.390**	1

**. Correlation is significant at the 0.01 level (2-tailed).

Discussion

Dengue fever is endemic in tropical and sub-tropical resource-poor countries including Pakistan. There is lack of availability and resources for diagnostic facilities and since outsourcing tests for diagnosis is a cumbersome and cost ineffective process, therefore Pakistan like many other developing nations, relies on an empirical clinical diagnosis

Several studies conducted worldwide have shown that a correlation of communal routine investigations of blood consisting white cell count, platelet count, liver functions, c-reactive proteins and coagulation cascade are useful in determination of dengue infection in laboratory.[10,11,12]

Our study showed that both thrombocytopenia and low TLC were significant laboratory parameters with a positive predictive value of 88% in patients of acute dengue infection, both findings are in parallel to marrow suppression during acute phase of the disease.

In Pakistan No such study has been done that has calculated predictive value either on a single parameter or combined. Our study presented a similar pattern to a study by Ho et al which showed that the positive predictive value (PPV) for combination of leukopenia, thrombocytopenia, elevated AST and low CRP is 89.5%.[2] This study has used multiple parameters for a predictive value. Pakistan is a health resource and budget constraint country therefore we considered minimal parameters from the most advised and easily available test (CBC) this shall eventually benefit physicians for a prompt and cost effective aid in diagnosis.

Chen CH et al in 2018 revealed that The PPV of combination of leukopenia, thrombocytopenia, and elevated transaminase levels were 100% on day 2 and day 6-8 of acute dengue infection.[12] The researcher in this study however included only pediatric patients whereas our study considered wider age group from 5 years to 50 years. The combined PPV reported is much higher in this study mainly because their study considered a panorama of dynamic clinical course of dengue infection whereas our study was based on a single diagnostic test done on a random day. Furthermore method of detection of dengue in Chen Ch et al was by detecting non-structural protein NS1, reverse transcription-polymerase chain reaction of dengue virus, and by dengue-specific IgM seroconversion as well. NS1 picks up cases earlier than IgM. Ns1 detection along with the addition of parameters other than CBC may also have contributed to an increase PPV.

Mussso et al compared diagnostic features of zika and dengue for their clinical differentiation.[13] Though the pattern and purpose of their study was different but they also calculated the combined positive predictive value of certain laboratory parameters for acute dengue infection. The positive predictive value of the of leucopenia and raised AST for the diagnosis of dengue was 90%, though the study showed thrombocytopenia to be frequent in dengue patients but did not take this parameter into account while calculating the combined PPV.

A study undertaken in China aimed to identify predictive laboratory markers for early diagnosis of dengue infection.[14] The positive predictive value of leukopenia and thrombocytopenia for DENV infection were 96.9% and 93.0%, respectively. These values obtained are again higher than ours [66% and 81% respectively] as the Viral RNA was detected by real-time RT-PCR, and viral-specific NS1 antigen was detected using enzyme-linked immuno sorbent assay being more sensitive methods in picking up the disease in its earlier phase. More over the study was carried out in an epidemic where as our study was conducted in acute febrile illness in an endemic area.

The findings were consistent with a study done by Liu et al, which showed that prolonged APTT, leucopenia, thrombocytopenia and raised AST has a PPV of greater than 80% and is useful in evaluating the likelihood of acute dengue infection.[15]

In all the studies compared and discussed, one highlighting feature has been the difference in the number of parameters considered for positive predictive value. Though the study of more parameters may have increased the PPV and hence the validity but since we live in an under resourced setting, our prime motive was to take into account minimal number of parameters that are readily available even in the most remote clinical setting. This will not only aid physicians in narrowing down their differentials but also in selective diagnostic testing for highly suspected cases. Additionally this is cost effective and practically applicable in setting like ours.

Limitations of present study include a smaller sample size and NS1 antigen and viral nucleic acid detection is considered for earliest diagnosis of dengue fever while we included only IgM positive cases.

Conclusion

In patients with signs and symptoms of acute febrile illness particularly in post monsoon period, a blood count analysis is imperative in assisting earlier diagnosis of dengue fever especially in under resourced setting with limited diagnostic facilities. Dengue fever is endemic in Pakistan and its complications are dire therefore future work regarding its diagnostic and prognostic aspects for early detection and management is the need of the hour.

Time trend analysis of various parameters specially platelets, ANC, eosinophil count, hematocrit and chemical analytes such as AST (aspartate aminotransferase) etc. must be done for understanding of disease progression, early recovery and timely management of any complication. Various new and novel parameters such as IPF (Immature platelet fraction) should be undertaken in the context of possible early diagnosis, monitoring of treatment or prognosis of acute dengue fever. Studies on larger sample size as well as with segregated data with respect to age group should also be conducted for better understanding of disease severity at different ages. Review Boards and Committees should be established for continuous assessment regarding diagnosis, treatment and prevention as well as control policies for dengue fever.

Conflict of Interest: None
Funding Source: None

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