

## Original Article

## Absolute Neutrophil Count and Thrombocytopenia in Patients of Acute Dengue Infection

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### Abstract

**Objective:** To determine mean Absolute Neutrophil Count and frequency of thrombocytopenia in patients of acute dengue infection.

**Methods:** This descriptive cross sectional study was done in Hematology Department, Medical and Pediatric wards, Fauji Foundation Hospital, Rawalpindi. A total of 100 patients diagnosed as a case of acute dengue infection based on 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 Hematology analyzer Sysmex XT 2000i system. Blood levels of Absolute Neutrophil Count and platelet count were recorded and documented for these patients.

**Results:** Out of 100 diagnosed patients of acute dengue infection, 81% exhibited thrombocytopenia, while the mean absolute neutrophil count calculated in these patients was  $1.96 \times 10^9 /L$ .

**Conclusion:** Acute dengue fever is characterized by thrombocytopenia and neutropenia. Therefore, in a patient with signs and symptoms of acute febrile illness, a blood count analysis is imperative in assisting earlier diagnosis of dengue fever.

**Key words:** Acute dengue infection, Complete Blood Count (CBC), Absolute Neutrophil Count (ANC), Thrombocytopenia.

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### Introduction

Dengue is an acute viral illness caused by dengue virus, and is a fast emerging mosquito-borne viral disease in humans, found in tropical and subtropical regions worldwide. Depending upon the epidemic activity, World Health Organization has estimated a global incidence of 50 million cases of dengue fever and several hundred thousand cases of dengue hemorrhagic fever per year.<sup>1,2</sup> The dengue virus comprises four different serotypes 1, 2, 3, and 4, which belong to an antigenic subgroup of the flaviviruses (Group B arboviruses). Any of these serotypes transmitted to humans initiates a spectrum of host responses, from an unapparent to severe and sometimes lethal infections.<sup>3,4,5</sup>

Early diagnosis of dengue during routine clinical care is challenging because the initial symptoms are often non-specific, viral load may be below detectable levels and the serological tests confirm dengue late in the course of illness. For adjusting appropriate management during the febrile stage, early diagnosis is essential.<sup>1</sup> But there

are several other infections which present with undifferentiated febrile illness like malaria, typhoid fever, leptospirosis, etc. The specific serological tests facilitate early diagnosis of most of the diseases but they are expensive and the inadvertent use of these tests escalates the total cost of the treatment.<sup>6,7,8,9</sup>

In the diagnostic workup of dengue patients, Complete Blood Count (CBC) holds an important place. Analysis of various CBC parameters in acute infection can assist the physician in better management of the patient.<sup>3</sup> Peripheral blood cells undergo a relative change in their percentage during illness and recovery of the disease. These findings direct that in the course of the disease, there are major shifts within cellular component of blood.<sup>3</sup> The most notable worldwide laboratory findings in patients of acute dengue infection include leucopenia, thrombocytopenia, prolonged APTT<sup>1,10,11,12</sup> and low Absolute Neutrophil Count (ANC).<sup>1,7,13</sup>

This study is directed towards the application of two parameters ANC and platelet count to evaluate acute

dengue infection, thus helping the clinicians in replacing the need of more costly serological investigations for diagnosis.

**Methods**

It was a descriptive cross sectional study. The study was carried out with permission from the ethical committee at Hematology 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 sum of 100 patients was involved in the study. Adults and children of both genders, age ranging from 5 to 50 years, admitted in pediatric and medical wards having acute dengue infection with no history of previous illness to remove any potential source of bias were enrolled. Patients who presented with signs of shock, having chronic illness at presentation or having an initial non dengue diagnosis were excluded from the study. The dengue infection was confirmed by serological test using an Indirect ELISA for anti DENV IgM. 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 was collected in CP bottle containing EDTA (ethylene diamine tetra acetic acid) as anticoagulant. It was analyzed on automated Hematology analyzer Sysmex XT 2000i system. ANC and platelet count were noted. The cutoff of thrombocytopenia in this study was  $< 150 \times 10^9/L$  and the cutoff. A proforma was designed in which blood levels of ANC and platelet counts 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 and thrombocytopenias were measured as frequency and percentages and quantitative variables i.e. age and ANC were measured as mean and standard deviation.

**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 adult patients was 28.5 years while that of pediatric patients was 9.4 years. A summary of the age statistics is shown in table 1.

Out of 100 patients 50 were males, and 50 were females as shown in figure 1.

Overall, 81 out of 100 patients had thrombocytopenia while 19 patients had normal platelet counts as shown

in figure 2.

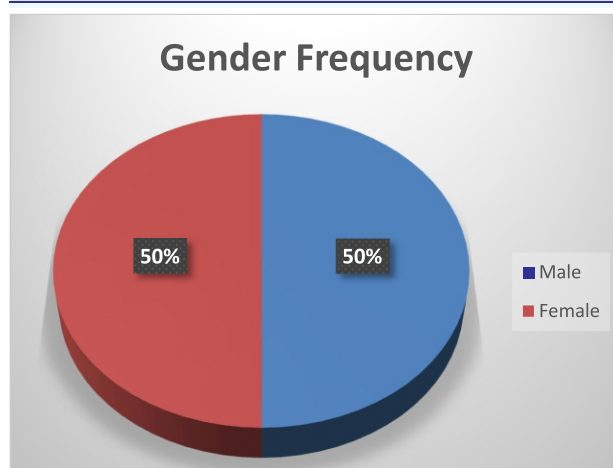
The frequency of thrombocytopenia in male versus female relative to age group is shown in table 6. Thrombocytopenia was most frequently observed in the age group of >10 to 20 years in both genders, while an additional peak was found in females in age group >40-50 years also shown in table 2.

Absolute Neutrophil Count ranged between  $0.20 \times 10^9/L$  to  $6.13 \times 10^9/L$  with a mean value of  $1.96 \times 10^9/L$  as shown in table 3.

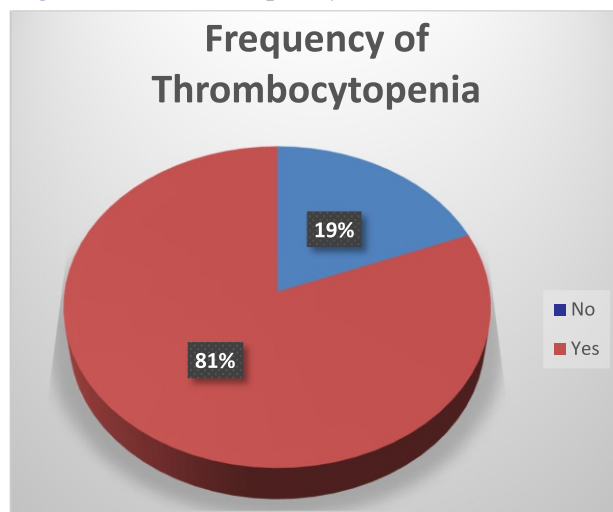
Population baseline characteristics are shown in table 4.

**Table 1:** Mean and Standard Deviation of age of dengue patients

| Age Descriptive Statistics |      |         |        |      |         |
|----------------------------|------|---------|--------|------|---------|
| Pediatric                  |      |         | Adult  |      |         |
| Female                     | Male | Overall | Female | Male | Overall |
| 8.5                        | 9.9  | 9.4     | 32.9   | 23.5 | 28.5    |



**Figure 1:** Gender Frequency



**Figure 2:** Frequency of Thrombocytopenia

**Table 2:** Frequency of thrombocytopenia relative to age group

| Age          | Male      |           |                | Female   |           |                |
|--------------|-----------|-----------|----------------|----------|-----------|----------------|
|              | 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             |
| <b>Total</b> | <b>12</b> | <b>38</b> | <b>50</b>      | <b>7</b> | <b>43</b> | <b>50</b>      |

**Table 3:** Mean and Standard Deviation of ANC in Dengue Patients

| ANC Descriptive Statistics |                 |          |          |        |                |
|----------------------------|-----------------|----------|----------|--------|----------------|
|                            | No. of Patients | Mini-mum | Maxi-mum | Mean   | Std. Deviation |
| ANC                        | 100             | .20      | 6.13     | 1.9600 | 1.33712        |

(Note: Values of ANC are expressed in 10<sup>9</sup>/L )

**Table 4:** Population Baseline Characteristics

| Population Baseline Characteristics |                           |
|-------------------------------------|---------------------------|
|                                     | Number of Participants    |
| Male                                | 50                        |
| Female                              | 50                        |
| Thrombocytopenia Present            | 81                        |
| Mean ANC                            | 1.96 x 10 <sup>9</sup> /L |

## Discussion

Dengue is an increasingly common arboviral infection affecting humans. The disease is endemic throughout tropical and sub-tropical resource-poor countries. The clinical spectrum of dengue illness can range from a self-limiting febrile illness i.e. Dengue Fever (DF) to severe disease forms like Dengue Hemorrhagic fever (DHF) and Dengue Shock Syndrome (DSS).

Classical dengue fever is characterized by fever, headache, myalgias, retro-orbital pain, arthralgias, vomiting and macula-papular rash. In Pakistan, the incidence of dengue fever is 50 to 100 million cases, associated with an overall mortality of 2.5%, each year. In Pakistan, like many developing nations with endemic malaria, an empirical clinical diagnosis is usually made, owing to limited resources and availability of diagnostic facilities.<sup>14,15</sup>

Early diagnosis of dengue infection would have an affluent impact on the distribution of health resources in endemic countries like Pakistan. It not only guides appropriate management, but can also decrease the

economic burden of dengue illness by prompt dengue control interventions.<sup>16</sup>

The confirmatory diagnostic tests for dengue infection include, virus expressed NS1 antigen detection, viral nucleic acid detection through PCR, serological diagnosis through high serum IgM and IgG levels and Hemagglutination inhibition (HI) test.

In a country like Pakistan, where we have limited diagnostic facilities and resource constraint hospital settings, outsourcing of diagnostic tests is time consuming and can delay early diagnosis and timely management of patients.

Keeping this in view in our study, we have tried to bring out the diagnostic utility of blood complete picture in patients presenting with signs and symptoms of dengue fever using two parameters i.e. Absolute Neutrophil Count and platelet count to evaluate the possibility of suspected disease. These tests are not a replacement for serological tests however, they can act as early presumptive diagnosis for this life threatening illness and also in resource poor setting where there is non-availability of kits, it can guide the physician in reaching a probable diagnosis. Thrombocytopenia usually occurs on 3<sup>rd</sup> day until 7<sup>th</sup> day of illness and reaches normal level on the 8<sup>th</sup>/9<sup>th</sup> day, while neutropenia occurs on 4<sup>th</sup> day of illness.<sup>17</sup>

Both thrombocytopenia and low mean ANC were found to be significant laboratory parameters in patients of acute dengue infection in our study. Both findings are in parallel to marrow suppression during acute phase of the disease.

The results of our study correlate with many studies that have been conducted locally as well as internationally regarding dengue fever and its various laboratory and clinical aspects. A study conducted by Tahir et al in 2012 discussed changing hematological parameters in dengue fever.<sup>3</sup> Their study was done with wide range of parameters, while ours was narrowed down to two hematological parameters, however they were comparable to the aforementioned study. In this study frequency of thrombocytopenia was 61%. This is marginally lesser than in our study. This variation can be most likely attributed to inclusion of all the cases i.e. acute phase as well as convalescence phase whereas our study was conducted only on acute dengue cases. In context of acute stage of dengue infection this study did not discuss neutropenia specifically.

Yogeesha et al in his study "Clinical and Laboratory Parameters Differentiating Dengue from Other Causes of Acute Febrile Illnesses in A Tertiary Care Centre in South India" in 2014 discussed various parameters that could assist in differentiating dengue from other febrile illnesses.<sup>6</sup> The study was conducted on 80 dengue

confirmed patients. The study showed thrombocytopenia and leucopenia to be 86.25% and 56.25% respectively in dengue positive patients. These results are similar to our study. However his study did not comment on neutropenia in these cases specifically while our study showed mean Absolute Neutrophil Count of  $1.95 \times 10^9/L$ .

Dsouza et al in 2013 conducted a study "Comparison of clinical and laboratory characteristics between children and adults with dengue".<sup>10</sup> He included patients with age range of 0-14 years as children and all patients of 15 years and above as adults. According to his study, Adults who were 15–19 years of age and children were the most affected groups in terms of highest frequency of thrombocytopenia and leucopenia. Thrombocytopenia was found to be 32.6% in children while it was 52% in adults. Our study showed thrombocytopenia to be most frequent in age group >10-20 yrs. in both genders however, a second peak of thrombocytopenia was found only in females in age group >40-50yrs. The reason for this peak has been attributed to female entitlement in our hospital setting, however their study showed no gender bias. Thrombocytopenia frequency seen in his study is moderately lower than our study. This might be due to inclusion of dengue positive cases through ns1 antigen. Our study in contrast included IgM positive cases only. NS1 antigen testing picks up infection in its earliest phase. The chances of having normal or better platelet count is more in earlier stages of infection, therefore leading to a lesser frequency of thrombocytopenia in this study.

Similarly Kalyanarooj et al in his study in 1997 brought out several parameters as indicators of acute dengue infection.<sup>18</sup> The study focused on total white cell count (TLC), Absolute Monocyte Count (AMC) and Absolute Neutrophil Count (ANC) in dengue patients in acute as well as convalescent phase. In observation phase after diagnosis of dengue infection, the mean ANC was found to be low i.e. 2.619/microliter. This low ANC is in congruence with our study. However, the study though reported cases with thrombocytopenia but has not commented on the frequency/ percentage in dengue infection.

In an another study conducted in Srilanka by Reller et al in 2012 showed mean platelet value in acute dengue patients to be  $190 \times 10^9/L$  and a median ANC of 3,600/microliter.<sup>7</sup> Though our study focuses on mean ANC and frequency of thrombocytopenia and has not considered the aforementioned parameters, this study exhibits thrombocytopenia to be an infrequent finding which is in contrast to our study. Our results however correlate their finding of thrombocytopenia in DHF (dengue hemorrhagic fever) patients. The difference may be ascribed to a larger sample size, wider age group and inclusion

of IgG positive cases along with IgM positive ones.

In a study conducted in Karachi, Pakistan by Hasan et al in 2013 the clinical spectrum as well as lab parameters in acute dengue fever were observed.<sup>19</sup> The study showed thrombocytopenia to be 31%. This is strikingly less as compared to our study. The possible reason can be the strict cut off criteria used for labeling thrombocytopenia i.e. <50,000 whereas in our study we have taken all cases below 150,000 as thrombocytopenia. Mean Absolute Neutrophil Count (ANC) has not been taken in account in this study however, leucopenia in dengue infection was reported to be 34.12%.

Thein et al, in a study conducted in 2014 studied trends of neutropenia throughout the course of disease.<sup>20</sup> The study was conducted on large sample size with longer time span. They categorized neutropenia as mild moderate and severe whereas ANC of >1.5 was included under the heading of others. According to this study during the hospital stay of patients with dengue fever, 82.2% of patients had some form of neutropenia. The study did not comment on mean ANC. However our study showed low mean ANC in patients of acute dengue infection. The values are relatable though not congruent, mainly because our study was a one-time analysis of mean ANC done randomly on IgM positive cases whereas this study assessed detailed time trends analysis for daily ANC.

Chatterjee N et al in his study "An observational study of dengue fever in a tertiary care hospital of eastern India" in 2014 discussed various clinical and laboratory parameters in acute dengue fever.<sup>21</sup> This study outlines the clinical spectrum as well as geographical expansion of the disease beyond urban confines. The study showed the disease affected both the genders equally and mostly the younger age group from rural as well as urban areas. His study showed thrombocytopenia to be 55% while leucopenia to be 32.7%. No result regarding mean ANC was reported.

Limitations of this study include that this study was conducted on a smaller sample size, Ns1 antigen and viral nucleic acid detection is considered for earliest diagnosis of dengue fever while we included only IgM positive cases, less parameters were taken into account for the disease evaluation and this study lacked the serial monitoring of disease progression through laboratory tests.

## Conclusion

Acute dengue fever is characterized by thrombocytopenia and neutropenia. Therefore in a patient 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.



### Future Recommendations

- 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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