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Correlation of Serological Markers with Haematological Parameters in Diagnosis of Dengue: A Single Centre Study at a Tertiary Care Hospital of Pakistan

About the Author(s)

Ayesha Sarwar[1], Maryam Zulfiqar[2], Asim Hanif[3], Maliha Saad[4], Muhammad Ashraf[5], Ashok Kumar Tanwani[6]

[1]Associate Professor, Department of Pathology, HBS Medical & Dental College, Islamabad
[2]Senior Lecturer, Department of Pathology, HBS Medical & Dental College, Islamabad
[3]Demonstrator, Department of Pathology, HBS Medical & Dental College, Islamabad
[4]Assistant Professor, Department of Pathology, HBS medical and dental college, Islamabad
[5,6]Professor, Department of Pathology, HBS Medical & Dental College, Islamabad

*Correspondence: ayeshaali1019@gmail.com
HBS Medical & Dental College, Islamabad

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Abstract

Objective: To correlate the serological markers with hematological parameters in the diagnosis of dengue fever.

Background: Dengue is a viral disease spread by Aedes Aegypti mosquitoes. It is most common in Southeast Asia. In recent times yearly epidemics of dengue fever has put a great burden on our health care system. The World Health Organization (WHO) has classified symptomatic dengue as dengue fever with or without warning signs and severe dengue. Early diagnosis of the disease and management can save countless lives in this curable infectious disease.

Methodology: It is a prospective observational study from HBS General Hospital, Islamabad. 102 patients who presented with fever and tested positive for NS1 antigen and/or IgM dengue antibodies and fulfilled the inclusion criteria over a period of one year were included. The demographic data along with serological and hematological results were recorded.

Conclusion: The most common serological test in dengue infection is NS- 1. Thrombocytopenia, leucopenia, monocytosis and reversed neutrophil lymphocyte ratio are important hematological clues in diagnosis of dengue fever. Thrombocytopenia is most severe in NS- 1 postive group as compared to antibody positive groups.

Key words: Dengue fever, hematological profile, thrombocytopenia, leucopenia.

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Introduction

Dengue virus measures 50 nm and has 11kb long genome. It is an sRNA virus. It contains three structural genes which encode capsid protein, membrane protein, and envelope protein. It also contains seven nonstructural genes which encode seven types of proteins. It is a mosquito borne viral infection and Aedes aegypti and Aedes albopictus families of mosquito are its vectors.[1] Dengue infection usually runs its course but some cases can be life threatening and mortality has been frequently reported.[2]

Dengue infection has been declared as the most rapidly spreading mosquito borne disease. The global incidence of dengue infection has significantly increased and a 30-fold rise has been reported in the last 50 years. Not only the incidence but a changing trend in the disease distribution is also noted in the recent years. The disease seems to have spread to new countries and more cases has been reported from the rural areas than before.[3] approximately every year about 50 million cases of dengue infection are reported with almost 2.5 billion at risk population reside in dengue endemic countries. Almost 70% of cases are from Asia. The disease is endemic in more than 100 nations. The increasing trend is evident from the fact that 44,585 cases were reported from India in 2020 and in two years this figure has risen to 193,245 cases in 2022. It means the prevalence of dengue fever increased more than three times in India. Similarly, Pakistan and Bangladesh has reported a seven and nineteen times, respectively increase in the cases of dengue fever in the last two years.[4]

In laboratory dengue virus infection can be confirmed by identifying the virus, viral nucleic acid, antigens or

antiviral antibodies.[5] The most commonly employed test for dengue viral diagnosis in first 4–5 days is detection of viral antigen. In 50% of patients IgM antibodies become detectable by days 3-5 of the diseases. Their levels steadily increase to 80% by day 5 and by day 10 th IgM antibody can be detected in 99% of the cases. The peak level of IgM antibodies is detected about two weeks after the onset of symptoms. After that it gradually decrease to undetectable levels in the next 2–3 months. Low titers of serum IgG becomes detectable at the end of the first week of illness and then they increase thereafter. The serum IgG is detectable after several months, and probably even for life.[6,7]

Many diagnostic methods are available for DENV detection like Polymerase Chain Reaction (PCR), dengue NS1 and antibody detection by enzyme-linked immunosorbent assay (ELISA) or rapid diagnostic test (RDT).[8]

In dengue fever the hematological parameters vary every day.[9] In this study we aim to correlate the serological markers with hematological parameters in dengue fever which will facilitate in the diagnosis of dengue infection, its risk categorization and will aid in timely management via the use of rapid serological tests.

Methodology

This cross-sectional observational study was conducted at HBS General Hospital, Islamabad. 102 seropositive dengue patients from the year 2022 were enrolled in this study. Nonprobability consecutive sampling technique was used.

Inclusion Criteria:

Male and female admitted patients of all ages with positive dengue serology tests. (Patients with serologically dengue NS1 antigen and/ or dengue IgM antibody positive result).

Exclusion Criteria:

1. Patients with other hematologic diseases, chronic diseases.
2. Patients who are on chemotherapeutic regimens and who are immunocompromised.
3. Patients with fever but no diagnosis has been established.
4. Dengue-infected patients with evidence of harboring another infection.

Admitted patients presenting to HBS hospital, fulfilling the inclusion criteria, were enrolled in the study after taking approval from the hospital's ethical committee. The study details were discussed with the patients and both verbal and written consent was taken. Most cases were admitted on the third day of the onset of the fever. 3ml of venous blood was collected in an EDTA tube by a clean venipuncture using a disposable syringe to analyze blood counts in a fully automated hematology counter (Sysmex Kx21/Medonic), while centrifugation was done at 3000rpm for 10 minutes. The hematological parameters from the daily CBC reports were recorded. The hospital stay for the admitted patients was 4 days minimally. After which the number of patients decreased each day due to discharge and day 7 data is available for 25 admitted patients who were initially enrolled in the study. NS- 1 and IgM antibody testing was done by rapid test method.

All data was entered and analyzed in SPSS version 21. Frequency and percentage were calculated for the qualitative variables like gender etc while mean and range were calculated for quantitative variables such as hemoglobin, hematocrit, WBC count, and differential leucocyte count. Data is represented as figures and tables.

The confidentiality and privacy of the patients was insured by not disclosing their personal information to anybody but those who are directly involved in this research project.

Results

A total of 1743 cases were diagnosed as dengue infection in 2022 on the basis of positive serology, out of which 102 patients were enrolled in this research project. There were 70 (64%) males and 32 (29%) females. Table1 shows the age groups of the participants.

Table 1: Age distribution of the subjects (n= 102)

Age group	Frequency (percentage)
10-20	4 (3.9%)
21-30	14 (13.7%)
31-40	18 (17.6%)
41-50	35 (34.3%)
51-60	19 (18.6%)
61-70	0 (0%)
71-80	3 (2.9%)

57.9% (59/102) patients tested positive for NS1 antigen, 27.2% (28/102) were dengue IgM antibody positive while both tests came out positive in 14.9% (15/102) patients. Out of 102 (12.7%) dengue positive cases, 13 were of severe dengue infection. The common presenting complaints were headache, nausea, vomiting, loss of appetite, rash, arthralgia and myalgia. Gastrointestinal symptoms like diarrhoea, abdominal pain and sore throat were also common.

The rise and fall of the several hematological parameters were observed over a period of 7 days after admission. Most cases were admitted on day 3 of the onset of the fever. Mean values of all haematological parameters were calculated according to the day of fever and findings are represented in the Table II

Table II: CBC Parameters according to the day of admission.

		Complete blood count (CBC) Parameters			
Day of fever	**No of admitted patients**	**Leukopenia (<4x10^3/uL)**	**Thrombocytopenia (<150x10$^{3/}$ µl)**	**Neutrophil count (<50%)**	**Monocytosis**
3	102	38 (37.2%)	102 (100%)	25 (24.5%)	23 (22.5%)
4	102	50 (49%)	102 (100%)	37 (36.2%)	31 (30.3%)
5	102	56 (54.9%)	102 (100%)	56 (54.9%)	45 (44.1%)
6	102	53 (51.9%)	102 (100%)	87 (85.2%)	57 (55.8%)
7	71	44 (61.9%)	71 (100%)	52 (73.2%)	47 (66.1%)
8	44	38 (86.3%)	44 (100%)	42 (95.4%)	29 (65.9%)
9	25	23 (92%)	25 (100%)	11 (44%)	14 (44%)

Table III: Correlation of serological markers with the haematological parameters in 102 serologically dengue positive cases.

Haematological parameter	**NS 1 positive N= 57.9% (59/102)**	**IgM positive N= 27.2% (28/102)**	**NS 1+ IgM positive N= 14.9% (15/102)**
TLC	4.9 (2.0- 8.4)	4.3 (1.3-8.4)	3.6 (1.7- 6.2)
RBC	4.5 (4.0- 6.2)	5.4 (4.0- 11.2)	5.4 (5.3- 5.6)
HB	13.2 (11.5- 14.4)	14 (12.9- 16.2)	12.7 (12.1- 13.6)
HCT	39.8 (35- 47.7)	44.6 (33.1- 48.8)	41.2 (31- 39.6)
Platelets	61.4 (9.8- 110)	84.6 (45- 183.6)	81.1 (42.8- 119.3)
Neutrophils	37.8 (29- 48)	51.4 (45.3 67.8)	42.6 (32- 55)
Lymphocytes	49.9 (36- 67.8)	32.4 (26.2- 41)	52.3 (49.8- 75)
Monocytes	8 (3.4- 6.6)	11.9 (3.4- 20)	6.2 (3.8- 10)
Eosinophils	4.4 (2.6- 7.0)	4.6 (2.5- 8.0)	3.3 (2- 6)

13 out of 102 (12.7%) dengue infected patients were classified as severe dengue infection and presented with symptoms like abdominal pain, bleeding and persistent vomiting. All enrolled patients were admitted for a period of 4 days. After which 26 were discharged and 5 were referred to other hospitals. By day 9 there were 25 admitted patients. The enrolled patients were divided in three groups according to their serology as NS-1 positive, IgM positive and NS- 1+ IgM positive. The mean values of each haematological parameter of all the admitted days was calculated and is represented along with range for that parameter in each group in Table III.

Discussion

Symptomatic dengue infection in most cases presents with low to moderate grade fever. But in almost 5% cases the disease is life-threatening disease and it is classified as “Severe dengue”. In the last fifty year the global incidence of dengue fever has significantly increased and it poses a substantial health and economic burden.[10]

In the present study most cases were of the age group was 41-50 years (34.3%) with 70 (64%) male patients and 32(29%) female patients. Similar demographic profile is reported by Qamash et al in the district Swabi of Pakistan with more cases in males and the most common age group involved was 21-30 years.[11]

Most patients were diagnosed with dengue infection because of positive NS 1 serology i-e 59/102 (57.7%). Habib MB et al have also reported a frequency of 77% positive NS-I cases with a male predominance and the most commonly affected age group in their study was 31-45-year.[12]

The hematological parameters studied showed one consistent feature and that was thrombocytopenia and leucopenia, which was present in all patients throughout the study duration i-e 7 days. A rising trend of platelets was observed from day 6, and the recovery of platelets occurs before a rising trend is seen in TLC. In our study a reversed neutrophil to lymphocyte ratio was observed from day 6 to 8. Monocytosis was present in > 60% of the cases in our study. Diseases like other viral infections, typhoid fever, tuberculosis, malaria, HIV infection, malignancy or pyrexia of unknown origin are also associated with monocytosis. It is not specific to dengue infection. Its cause is believed to be the monocytes and macrophages involved in phagocytosis and antigen presentation to T helper cells.13 Sivathanu et al have reported monocytosis in 91% cases of dengue infection in their study on 67 children.[14]

Chaloemwong J study from Thailand in 2018 also reports that thrombocytopenia and leucopenia in dengue patients in the start of fever with recovery of these

parameters from day 5 of the fever. They have reported a higher monocyte percentage from day 1 to 4 and neutrophil to lymphocyte ratio was noted to reverse on day 6 to 9 of fever in their study.[15]

The role of non structural antigen NS1 is pivotal in the pathogenesis of dengue infection. It causes vascular leak through binding toll like receptors and damaging the endothelial glycocalyx. Some studies show the role of NS 1 in activating compliment membrane attack complex C5b-C9 on the endothelial cells to destroy viral infected cells but at the same time it cause release of anaphylotoxins like C3a- C5a which promotes inflammation.[16] The human immunity has an important role in the pathogenesis of the disease. Dengue infection is most severe when the virus is being cleared by the host immune system and not with the peak viral load. Some antiNS1 antibodies can cross-react with endothelial cells and cause apoptosis, thus creating a form of autoimmune response. Anti NS1 antibody also causes endothelial cells to produce IL-6 and IL- 8, both of which are found to be elevated in patients with severe dengue infection.[17] In our study the hematological parameters did not differ much in the three serological groups. The IgM positive group showed maximum hematocrit and hemoglobin levels. Antigen as well as antibody takes part in endothelial cell damage and plasma leakage which in turn causes hemoconcentration; an important hallmark of dengue infection. Dengue virus has an avidity to infect hepatocytes. Hepatocyte apoptosis releases soluble substances in blood which also causes consumption deficiency of platelets. Most severe thrombocytopenia in our study was seen in the NS- 1 positive groups. (mean value 61.4x103/uL compared to 84.6x103/uL and 81.1x103/uL in other two groups)

A large scale study conducted in Nepal in 2023 analyzed the serological, biochemical and hematological parameters in dengue infection. Their results are comparable to our study. The hematological parameters did not differ much in the three groups. The NS -1, IgM positive and NS- 1+ IgM positive groups showed thrombocytopenia and leucopenia. But monocytosis was seen in NS- 1 positive group mostly which in our study is in IgM positive group. Same goes for lymphocytosis which in our study is more pronounced in NS- 1 and IgM positive group than the other groups.[18] A study in 2018 by Joshi A et al showed highest values of Hb, and RBC count in cases with positive antibody as compared to antigen alone. But in contrast to our study thrombocytopenia in their study was more severe in antibody positive cases than NS- 1 positive cases.[19] In contrast to our study, Mishra et al showed lowest platelet counts in patients who are NS- 1+ IgM positive.[20]

It is currently not clear if anti NS1 antibodies against different regions of NS1 antigen play roles in the defense or disease progression of dengue fever in humans. According to the current available data it seems that multiple factors which include viral load, patient immune status, NS1 antigen, anti-NS1 antibodies, infecting serotype, and genotype may all contribute in the pathogenesis of dengue infection and the development of severe disease. The nature and mode of these interactions is not fully understood yet and there are many grey areas.

Conclusion

The most common serological test in dengue infection is NS- 1. Thrombocytopenia, leucopenia, monocytosis and reversed neutrophil lymphocyte ratio are important hematological clues in diagnosis of dengue fever. Thrombocytopenia is most severe in NS- 1 positive group as compared to antibody positive groups.

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Authors Contribution:

[1,6]Substantial contributions to the conception or design of the work;
[2,3,4,5]the acquisition, analysis, or interpretation of data for the work & Final approval of the version to be published
[1,6] Drafting the work or revising it critically for important intellectual content.