

EVALUATION OF SPECTRUM OF LABORATORY HEMATOLOGICAL MANIFESTATIONS IN DENGUE FEVER

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ABSTRACT

Dengue virus belongs to flavivirus family which gains entry into the host organism through skin following an infected mosquito bite. Humoral, cellular, and innate host immune responses are involved in the progression of the disease. Dengue fever is getting common in Pakistan, and at times shows high mortality, but there is limited literature available. Therefore, this study was designed to evaluate hematological parameters in patients with dengue fever. This was a retrospective cross-sectional study conducted in the Department of Microbiology, Dow Diagnostic Reference and Research Laboratory, Karachi, Pakistan from 1 January 2021 till December 2021. A total of 6140 were collected, out of which 1746 were found positive. Dengue infection was confirmed by rapid screening NS1 antigen by ICT method. IgM antibodies were detected by Enzyme linked immunosorbent assay (ELISA). Hematological analysis was performed on Sysmex analyzer. Among the 1746 positive samples, 1036 (60%) were males and 710 (40%) were females. More than 10 hemoglobin and 45 hematocrits were found in greater than 40% of cases. Leucopenia less than 4000 was observed mostly in age group 0-20 years. Eosinophilia, basophilia, lymphocytosis and atypical lymphocytosis were shown equally in all age groups. Our study found greater incidence of Dengue

fever among 21-40 years of age group with male predominance. Hematological spectrum revealed thrombocytopenia, lymphocytosis, high hematocrit, eosinophilia, basophilia and monocytosis at the time of diagnosis.

Key Words: Dengue fever, Incidence, Hematological parameters

INTRODUCTION

Dengue virus is a member of flavivirus family which gains entry into the host organism through the skin following an infected mosquito bite. Humoral, cellular and innate host immune responses are involved in the progression of the illness and the more severe clinical signs occur following the rapid clearance of the virus from the host organism. It has an acute onset and is often self-limiting in most cases(1). According to the World Health Organization (WHO) report dengue has emerged as a major global public health challenge especially in the tropic and sub-tropic nations with almost 30-fold upsurge worldwide between 1960 and 2010, due to increased population growth rate, global warming, unplanned urbanization, inefficient mosquito control, frequent air travel, and lack of health care facilities providing unhindered opportunities for mosquito breeding(2). The clinical presentation of dengue fever is tri-phasic with the febrile phase typically characterized by high grade fever, headache, myalgia, body ache, vomiting, joint pain, transient rash and mild bleeding manifestations such as petechiae, ecchymosis at pressure sites and bleeding from venipunctures (3). In the next critical phase, there is a heightened risk of progression of the patient to severe dengue which can result in shock or fluid accumulation such as ascites or pleural effusion with or without respiratory distress, severe bleeding, and/or severe organ impairment. The risk of severe bleeding in dengue is much higher with a secondary infection and is seen in about 2-4% of cases. Atypical presentations are also encountered with acute liver failure, encephalopathy with seizures, renal dysfunction, lower gastrointestinal bleeding. Several studies have previously analyzed the clinico-epidemiologic profile of dengue infection (4). To differentiate

them on clinical findings is troublesome and to reach a definitive diagnosis on the basis of serology is difficult in the areas of limited resources. NS1 Antigen, IgM antibodies and complete blood examination is a routine initial investigation performed at many laboratories and most frequently suggested initial investigation by the physicians(5).

Hematological parameters in various situations are helpful in giving a clue about dengue and these parameters are also useful in the management of the disease. A few findings such as presence of plasmacytoid lymphocytes and thrombocytopenia are associated with dengue infection so these CBC parameters can be utilized as diagnostic clue in area where lab facilities are scarce and patient cannot afford to go for serology. In addition evaluation of other CBC parameters can be evaluated for a potential help in diagnosis of dengue(6).

Therefore, this study was designed to evaluate different hematological parameters in samples of patients with confirmed diagnosis of dengue fever. The CBC findings as identified in this study will serve as a surrogate marker in helping the clinicians to diagnose dengue less developed settings.

METHODS

This was a retrospective study conducted at the Department of Microbiology, Dow Diagnostic Reference and Research Laboratory, Karachi, Pakistan. All patients who presented with acute fever from 1st January 2021 till December 2021 were included in this study.

Collection of clinical samples: A total of 6140 blood samples were collected from different areas of Karachi in collaboration with Dow Diagnostic Reference and Research Laboratory. All these patients had history of fever of acute onset. A 5ml venous blood was received in yellow gel tube then tubes were centrifuged for 10 minutes and the serum was separated into another tube and tested for immunoglobulin M (IgM) serum dengue antibodies.

Screening for dengue antigen/ antibodies: Currently the two serological tests are being used in the laboratory to confirm the diagnosis of dengue. The detection of NS1 dengue antigen, its sensitivity is 76% and specificity is 98% and another one is dengue IgM antibodies by Enzyme linked immunosorbent assay (ELISA), it reported specificity is 93% and sensitivity is 90% (23). Initially, the diagnosis of dengue infection was confirmed by rapid screening NS1 antigen was detected by ICT method using NS1 dengue antigen kit. It's the rapid qualitative dengue virus detection test which can be done from both serum and plasma. Further, confirmation was done by detection of antibodies in serum through IgM a micro titer plate technique. This technique identify IgM antibodies through ELISA. In this techniques 96well plates were used, coated with specific antigen for dengue antibodies. After antigen antibody's reaction the color changes that was read on ELISA reader. The absorbance of every sample was noted and compared with cut off values to differentiate positive and negative tests for dengue.

Hematological analysis: Hematological analysis was performed on Sysmex XN-1000 which is calibrated after every six months and the quality control done on regular basis. 2ml of blood collected into EDTA containing purple top tubes. Complete blood count was done by Sysmex analyzer and different parameters such as hemoglobin (Hb), hematocrit (HCT), white blood cells count, differential percentages of the white blood cell counts and platelets. Sample was loaded on the analyzer which displays their result on screen. All the results were recorded.

Statistical analysis

The data was analyzed using Statistical Package for Social Sciences (SPSS) version 25.0. Demographic data and laboratory data were presented as descriptive statistics including frequency distribution and percentages.

RESULTS

A total of 6140 samples were received for the dengue antigen test in which 1746 (28%) were positive, 4709 (76.6%) were negative and 634 (10.28%) samples were rejected due to errors. The patients were both males and females ranging from 2 months to 104 years of age. Among the 1746 positive samples, 1036 (60%) were males and 710 (40%) were females (Figure1).

In this study, there were 471 patients of age group from 0 years to 20 years in which hemoglobin level below and above 10 was seen in 196 (41.6%) and 275 (58.38%) patients, respectively with Hematocrit above 45% was observed in 287(60.93%) patients. Leucopenia was most commonly seen in the age group 0 years to 20 years in 206 (43.73%). Lymphocytes were increased above 45 was detected in 252 (53.5%) patients whereas atypical lymphocytes were seen in 398(84.5%).

In age group between 20 to 40 years 853 patients were positive for Dengue antigen, hemoglobin level below 10 and above 10 was seen in 337(40%) and 516 (60.49%) of patients respectively. In the age group 21-40 (n=853) 620 (72.6%) of patients' showed hematocrit above 45. WBCs were investigated below 4000 in 152 (17.8%), and >11000 in 280(32.8%) patients. Lymphocytes above 45 and atypical lymphocytes were evaluated in 551 (64.5%) and 666 (78.07%) respectively. Dengue antigen was positive in 334 patients of ages between 40 to 60 years in which hemoglobin >5, between 5 to 10 and above 10 were detected in 127 (38%) and 207 (61.9%) respectively. In 218(65.26) of patients' hematocrit above 45 was seen. WBCs were evaluated above 4000 in 270 (80%), and >11000 in 64 (19.16%) patients. Lymphocytes above 45 and atypical lymphocytes were evaluated in 224 (67.06%) and 188 (56.2%), respectively.

In adult age group above 60 years, 88 patients were detected positive for dengue antigen in which hemoglobin level below and above 10 is seen in 50 (56.8%) and 38 (37.5%), respectively. In 23 (26%) of patient's hematocrit above 45 was seen. WBCs were perceived below 4000 in 15 (17%), and >11000 in 20 (22.72%) patients. Lymphocytes above 45 and atypical lymphocytes were evaluated in 52 (59%) and 61 (69.31%), respectively. A summary of hematological indices are shown in Table 1. Figure 2 shows platelet pattern in Dengue fever.

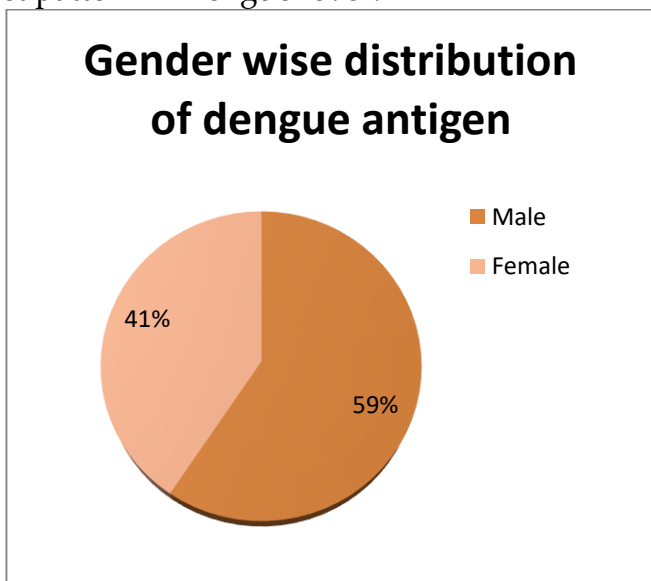


Figure 1: Gender wise distribution of dengue antigen. Light orange color shows the female percentage and dark brown shows the frequency of the males.

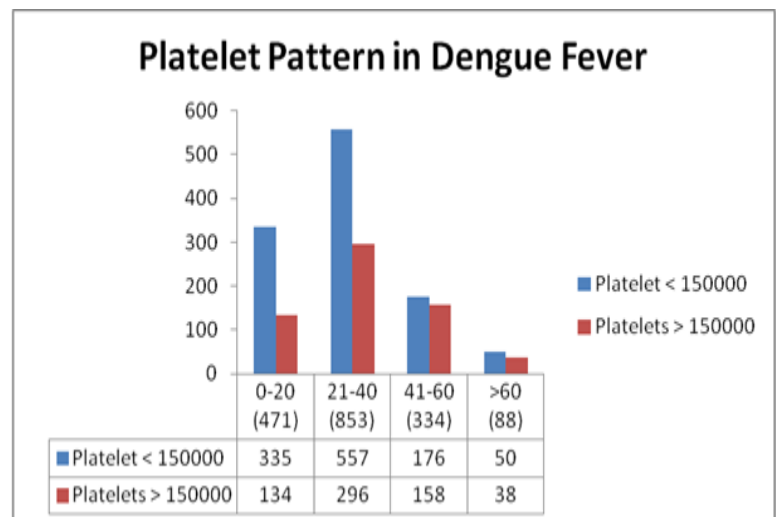


Figure II: Platelet shows thrombocytopenia in majority of patients of all age groups shown in.

Table 1: Hematological parameter (Hemoglobin, Hematocrit, WBCs, lymphocytes, atypical lymphocytes, Eosinophils and Basophils) among patients:

Age groups	Sample size	Hemoglobin >10 n(%)	HCT >45 n(%)	WBC <4000 n(%)	Lymphocytes >45% n(%)	Atypical lymphocytes n(%)	Eosinophils >5 n(%)	Basophils >2 n(%)
0-20	471	196(40)	287(60.93)	206 (43.73)	252(53.5)	398 (84.5)	15(3.18)	186(40)
21-40	853	220(40)	620(72.6)	152(17.8)	551(64.5)	666 (78.07)	119(13.9)	398(46.6)
41-60	334	66 (38)	218 (65.26)	41(12.2)	224(67.06)	188(56.2)	24(7.1)	169(50)
>60	88	50(56.8)	23 (26)	15(17)	52(59)	61(69.31)	7(8)	38(43)

DISCUSSION

Dengue is a mosquito-borne disease and is one of the major health threat (7). The fast rate of globalization, growing burden of population, poor waste disposal and accumulation of water result into breeding habitats for mosquitos resulting in increasing number of mortality and morbidity (8).

A dengue is a hemorrhagic viral fever with grave magnitudes and can turn out to be fatal. Hence, this study was designed to analyze laboratory dynamics in order to increase the likelihood of prompt diagnosis (9). Serological diagnosis of dengue virus infection is normally performed by observation of the NS1 antigen and dengue IgM antibodies in the patients. Our study reported male predominance and is in harmony with the other study reported previously(10). The incidence of dengue fever in the current study was higher in the group aged 21-40 years, which was consistent with a previous study by Rabbani et al (11). Hematologic parameters appear to be useful in differentiating dengue infection from other infections by examining the complete blood picture. The most important finding of present study is thrombocytopenia which is in agreement with other studies reported previously(12, 13). B lymphocytes are commonly infected by dengue virus. The excess production of B cells, accompanied by IL – 6 stimulate an abnormal development of plasma cells and atypical lymphocytes that further lead to the formation of autoantibodies against platelets resulting in thrombocytopenia. Presence of these anti-platelet antibodies also hinders ADP brought aggregation of platelets. Thrombocytopenia also results from infection and suppression of the bone marrow megakaryocytic precursors. It has been reported in the literature that overproduction of cytokine and atypical lymphocytosis are contributing factors to the pathogenesis of dengue fever (14).

Our study showed leucopenia, in line with the findings of other study (15). A hypothesis is related to the incidence of the leucopenia was due to the destruction of myeloid progenitor cells because bone marrow investigations exhibited initial hypocellularity then normal cellularity(6). Leukocytosis is uncommon finding usually associated with the superimposed bacterial infections.

Lymphocytosis was observed in current study especially higher in age group 40-60 years. The lymphocytes in the differential leukocyte count are good predictor of the length of hospital stay. The faster recovery and short duration of stay in the hospital is directly proportional to the higher lymphocyte count (16). Elevated lymphocytes with atypical morphological features have been observed in some studies, which is similar to our study (17). Atypical lymphocytes have increased amount of cytoplasm with basophilic cytoplasmic edges, scanty nucleoli may be found but non-neoplastic in nature(18). Severe dengue infection is related to elevated lymphocytes with atypical features, which specifies the role of immunological response, including cytokines production during initial and subsequent exposure of the disease (19). Moreover, exposure of secondary viral antigen

resulted in aggressive form of disease could be due to the amplified immune response ,also called as “the original antigenic sin”(20).

High hematocrit points towards plasma leakage from blood capillaries. Our study demonstrated greater than 45 hematocrit in all age groups which is in alignment with other study(21). Hematocrit values are quite helpful in monitoring amount of intravenous fluid and also direct us about the use of blood products if needed in dengue patients. The high hematocrit along with leucopenia and thrombocytopenia are substantial in the progress of severe dengue infection. Eosinophilia was observed in present study. Eosinophil count lowers in initial phase of study due to the inflammatory markers but it rises during the convalescent stage of diseases, also reported by other study (15). Monocytosis found raised in patients in our study, concomitant with the findings of study. Monocytes are mostly raised in dengue hemorrhagic fever, thus indicating the severity of disease. Moreover, it is speculated that monocytes increases in early phase of disease because they are a part of innate immunity helps in phagocytosis during the prodromal period (15). Basophilia specifies salvage from suppression of bone marrow during convalescence serves as a recovery marker in Dengue. It is usually seen in inflammation and myeloid leukemia. Current study observed basophilia, results are endorsed by other study(22). The study has presented hematological pattern of dengue in a large population. However further studies to confirm these findings will be required.

CONCLUSION

Dengue is one of the most important public health problems in resource limited countries like Pakistan. Our study found greater incidence of dengue fever among 21-40 years of age group with male predominance. Hematological spectrum revealed thrombocytopenia, Lymphocytosis, high hematocrit, eosinophilia, basophilia and monocytosis. The infection control practices, proper vector control, surveillance program and prompt diagnosis would be helpful in reducing mortality and morbidity.

ETHICAL CONSIDERATION: This study was approved by local Research Ethics committee.

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CONFLICT OF INTEREST: Authors declare no conflict of Interest

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