Section A-Research Paper
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Evaluation of Haematological Parameters in Dengue Cases and controls: A retrospective Study

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ABSTRACT

Background Dengue is a viral infection transmitted to humans through the bite of an infected mosquito. Various clinical and laboratory parameters are used to predict the severity of this disease.

Objective This study was aimed at comparing and analysing eight haematological parameters, including haemoglobin, haematocrit, platelet count, three platelet indices [mean platelet volume (MPV), plateletcrit (PCT), platelet distribution width (PDW)], total leucocyte and erythrocyte count, in the case of dengue seropositive, dengue seronegative but clinically suspected dengue and healthy controls.

Methods We retrospectively analysed patient records from the Central Laboratory of Subharti Hospital, Meerut (UP). In this laboratory based-study, between October 2021 and January 2022, 300 subjects were examined including 200 hospitalised patients with probable dengue fever and 100 healthy subjects.

Result The data was evaluated from 200 patients and 100 healthy controls (age between 6-70 years, mean 31 years). Quantitative analysis of patients and controls laboratory data revealed a significant difference in haemoglobin, packed cell volume (PCV), platelet count, and PDW (platelet distribution width) with p-values <0.05. Qualitative analysis of all-haematological parameters, was also statistically verified and had p value < 0.001.

Conclusion In addition to platelet count, haemoglobin, PCV, and PDW are useful parameters to assess severity and recovery after dengue diagnosis.

Keywords: Dengue fever, CBC, PCV, haemoglobin, platelet count.

INTRODUCTION

Dengue is the most common flavivirus in tropical and subtropical regions. [1,2] It is also a serious health problem in India. According to Global Model data, 33 million clinically detectable dengue cases occur in India annually, which is one-third of all dengue cases worldwide.[3] The primary vectors that transmit the disease are *Aedes aegypti* female mosquitoes and, to a lesser extent, *Aedes albopictus*. Positive-strand RNA viruses encode

extracellular matrix (ECM) proteins, membrane proteins, and nuclear capsid (DENV) proteins. The four serotypes of dengue virus are DEN-1, DEN-2, DEN-3 and DEN-4. [4]. The extrinsic incubation period in mosquito vector is 8-12 days, intrinsic incubation period is 3-14 days and period of infectivity is about seven days. [5]

Dengue presents clinically with fever, headache, retro-orbital pain and general weakness, with or without nausea and vomiting. Bone marrow suppression can be complicated by varying degrees of thrombocytopenia and leakage of vessels lined with dysfunctional endothelial cells, manifesting as dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Inflammatory cytokine production, including immune-mediated responses, is thought to be responsible for these life-threatening complications. Its diagnosis is based on clinical, epidemiological and laboratory data. There are non-specific laboratory tests (blood tests) and specific laboratory tests (isolation and serological tests to detect antibodies and antigens such as IgG, IgM and NS1). The gold standard for DF detection is DNA sequence-based amplification (NASBA), an RNA-specific isothermal amplification assay that does not require thermal cycling. Virus cultures are used, particularly *Aedes albopictus* C6/36 or AP61 (*Aedes pseudoscutellaris*). However, the NASBA method and virus culture require a lot of time, money and effort. Therefore, dengue diagnosis is based on card tests to save time and money. RT-PCR and ELISA have become the new standard due to its high specificity and sensitivity. [6] The aim of this retrospective study was to analyse whole blood variables such as haemoglobin, PCV, platelet count, platelet indices, total leukocyte count (TLC) and erythrocyte count in clinically suspected dengue cases and controls.

METHODS

Study design

This study was a laboratory based retrospective study was conducted in collaboration between the Department of Pathology, Subharti Medical College and the Central Laboratory – Department of Microbiology and Pathology, Chhatrapati Shivaji Subharti Hospital (CSSH), Meerut. Data was collected from all patients who had symptoms of dengue between October 2021 and January 2022. Two screening methods were used in this study, serological and haematological. A plane vial (red top without anticoagulant) and Potassium (K_3) EDTA anticoagulant vial (lavender top) was used to collect whole blood samples for serological testing and CBC testing, respectively.

Serological studies

The commercial kit (Aspen Dengue Combo Test) has often been used as a first step, which detects the NS1 antigen and IgM and IgG antibodies to the dengue virus. In primary and secondary dengue infection, NS1 antigen should be detectable 1 day after onset of fever and persist for up to 9 days. Measurable IgM is present 3-4 days after the onset of primary dengue fever. Secondary dengue fever is characterized by an IgG increase 1 or 2 days after the onset of infection, usually accompanied by an IgM increase. According to the product insert, the tests have a sensitivity of 98.01% and a specificity of 99.4% for diagnosis of dengue.

Haematological studies

Blood parameters are determined for dengue seropositive, seronegative and healthy controls. A Horiba haematology cell counter with 27 parameters and a 6-segment differential (Montpellier, France) was used for the Complete blood count (CBC). It includes Red Blood Cell Count (RBC), White Blood Cell Count (WBC) and Platelet Count. Analysers work on a combination of principles that are light scatter, electrical impedance, fluorescent light absorption, and electrical conductivity. Automated haematological analysers are widely employed in clinical laboratories to evaluate blood samples.

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Statistical analysis

Descriptive statistics was performed to compare the three categories (dengue positive, dengue negative, and healthy controls) using the R program. Qualitative and quantitative analyses, i.e., the t-test and the Kruskal-Wallis test, were performed.

RESULTS

The study had 300 participants, among whom 100 were positive for dengue on serological testing, 100 had manifestation of the disease but were serologically negative, and 100 were healthy controls. Both the serologically positive and clinically suspected, but serologically negative were taken as cases and compared with healthy controls. All the subjects included in the study were in the age range of 6 to 70 years. The age wise distribution of the total 300 dengue cases and controls is shows by table 1. There was slight male preponderance in all three categories, with 57, 55, and 52 males in the dengue serologically positive, clinically dengue suspect, and but serologically negative and healthy subjects, respectively. Out of 100 people who tested seropositive for dengue, had 52 NS1 antigens, 18 IgM antibodies, 12 IgM antibodies and NS1, 4 IgG antibodies and NS1, and 6 IgG and NS1. Only eight people had all three NS1 antigen, IgG, and IgM antibodies.

With the aid of the automated haematology analyser and the LIS (Laboratory Information System), data from participants and controls was gathered. Data on certain CBC parameters, including haemoglobin, packed cell volume, platelet count, platelet indices, total leucocyte count, and erythrocyte count, were included in this study.

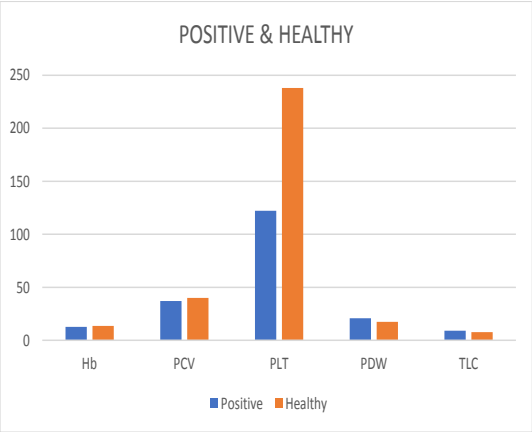
STATISTICAL ANALYSIS

The R programme uses descriptive statistics to analyse the collected data and compare the three groups. For quantitative analysis (comparison between dengue seropositive and dengue seronegative as well as healthy control), the T test was applied, and for qualitative analysis (comparison between all three categories), the Kruskal-Wallis test was applied and significant p value <0.05 was taken.

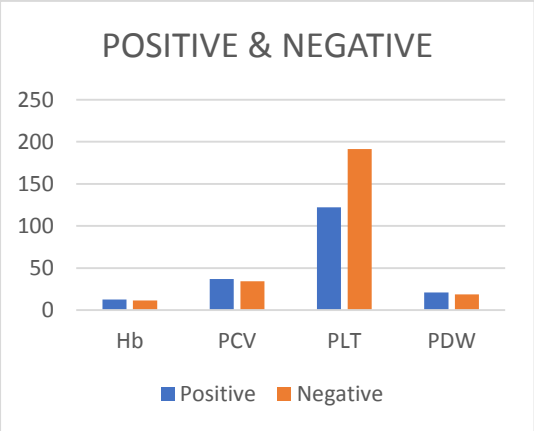
QUANTITATIVE ANALYSIS

When comparing dengue seropositive and dengue seronegative individuals, there is a significant difference in Hb, PCV, PLT, and PDW values, with p values of 0.002, 0.011, 0.000, and 0.005. And when we compare the parameters of dengue seropositive patients with healthy controls (graph no. 2), we see that there is a significant difference in haemoglobin, packed cell volume, platelet count, platelet distribution width, as well as total leucocyte count. The relevant p values for Hb, PCV, PLT, PDW, and TLC are 0.007, 0.002, 0.000, 0.000, and 0.026. This difference is represented graphically by the mean values (graph no. 1&2). The clinically suspect dengue seronegative cases when compared with healthy controls showed p values < 0.0001 for five parameters studied as the seropositive cases (data not shown).

"Figure 1: Mean value of seropositive and healthy"



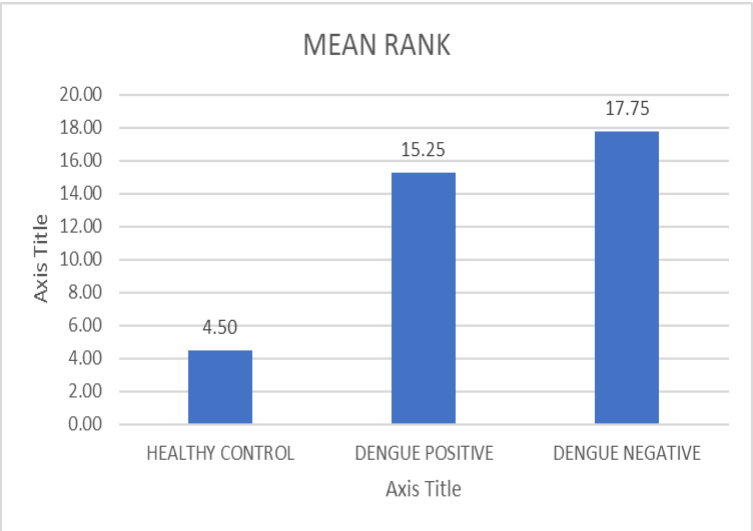
"Figure 2: Mean value of seropositive and seronegative"



QUALITATIVE ANALYSIS

The Kruskal-Wallis test was used for comparison of all three categories. In this, we only take the abnormal variables from 100 variables of each parameter in all three categories according to their age and gender. Its test value is 16.14 and its p value is <0.001, as the p value is less than 0.05, so it is statistically verified. The result is represented graphically by the mean values.

"Figure 3: Mean value of all abnormal parameters in three categories"



"Table 1: Age distribution of Cases and controls"

Age groups (years)	Serologically positive (100)	Serologically negative (100)	Controls (100)
≤15	25	18	15
16-30	29	32	35
31-40	15	19	15
41-50	11	10	12
51-60	12	10	11
61-70	5	6	8
71-80	3	5	4

"Table 2: Frequency of abnormal CBC parameters in dengue seropositive, seronegative and healthy control cases"

SN	CBC Parameters	Abnormal parameters in Dengue serologically positive cases	Abnormal parameters in Dengue serologically negative cases	Abnormal parameters in healthy controls
1	Haemoglobin	37	48	6
2	PCV	37	48	6
3	RBC	35	45	0
4	TLC	22	26	0
5	Platelet count	35	54	0
6	PDW	69	56	0
7	MPV	22	32	0
8	PCT	93	98	0

DISCUSSION

Dengue is the most significant viral disease transmitted by mosquitoes. Acute febrile illness is the typical presentation of traditional dengue fever. Dengue haemorrhagic fever (DHF), a more severe form of the disease, develops in a small percentage of cases. The severity of dengue fever can range from mild illness to dengue shock syndrome (DSS). Complete blood counts provide information about the possible aetiology, and serological tests are performed to arrive at a working diagnosis.

Demography

In the present study, the age of subjects varied between 6 and 70 years (median 28.5 years). A study reported a median age of 31 years in their study. Our results are concurrent with *Daumas et al*. [7]

The gender distribution of subjects revealed that there were 164 males (54.67%) and 136 females (45.33%), with a male to female ratio of 1.2:1. A male to female ratio of 1.2:1, with a male preponderance of 55.23% and 44.76% female, was reported previously. [10].

Red cell parameters

A study by Ambuja Kantharaj concluded that the most essential measure for detecting complications of dengue is HCT. Increased HCT is a sign of haemoconcentration and might indicate incipient haemorrhagic shock, which is found in dengue fever patients who are more susceptible to DSS. A reduction in HCT is a sign of haemorrhage and could indicate possible internal bleeding. This is more common in patients with DHF. [8] Another study reported that elevated haematocrit in both patterns, i.e., dengue haemorrhagic fever and dengue fever. [9] These studies show that dengue fever patients have higher haemoglobin and PCV/HCT levels, and our study was comparable to other studies.

Platelet indices

In our study, almost all dengue positive patients presented with thrombocytopenia and high PDW. PDW was increased in 69% of dengue seropositive and 56% of dengue seronegative patients (Bar graph 2). Bone marrow depression, increased destruction by anti-virus antibodies and platelet consumption coagulopathy could be possible aetiology of thrombocytopenia. Mohan *et al*. found both longer duration of illness and recovery from thrombocytopenia were linked to higher PDW (10). Navya *et. al* found that 92% of cases had high PDW, which could therefore suggest possibility of dengue and require serological confirmation. [11]

Leucocyte count

A study observed significant association of TLC with serological groups (p value <0.05) (7). In our study, we also reported a significant difference in TLC when we compared the dengue positive to healthy controls (p value 0.026), while both dengue seropositive and seronegative were similar.

One prospective study suggested that MPV and PDW alteration as a valuable marker for supporting a possible dengue fever.[12] Several studies stated that TLC as a valuable parameter to monitor and prognosticate dengue illness. [13-15] All except one were prospective, general properties of these studies are shown in Table 3.

CONCLUSION

Haematological parameters can be used to predict prognosis and are immensely useful for disease monitoring. Haemoglobin, PCV, and PDW-based transfusion choices may also assist to rationalise the necessity for red cell and platelet transfusions in dengue and enhancing the blood centre's readiness to offer the required blood components for transfusion. These parameters may be useful for providing improved care for complex situations if properly and promptly evaluated. It is recommended that future research evaluate the therapeutic importance and usefulness of the association between haematological and serological indicators in predicting and treating dengue patients.

AUTHORS' CONTRIBUTIONS

Dr. Mahendra N. Mishra and Dr. Umesh Kumar participated in the conception, design, and coordination of the study; Yashika Bhardwaj performed the study and drafted the manuscript.

CONFLICTS OF INTEREST

The authors report no conflicts of interest. In this study, data of the patients was collected from laboratories. Human participants were involved as it was a retrospective study, so no informed consent was involved.

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