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Original Research Article

A Hospital Based Observational Study to Assess the Association of Dengue Serology with Variations in RBC Parameters

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Abstract

Aim: The aim of the present study was to assess the association of dengue serology with variations in RBC parameters.

Methods: The present study was conducted in the Department of Pathology. Out of 200 total study populations, 100 were dengue positive and 100 were dengue negative.

Results: Multiple logistic regression showed thrombocytopenia, leukopenia (ORA = 0.999; $p < 0.001$), glucose level, aspartate aminotransferase and monocytosis as significant parameters in the NS1-only positive group. Similarly, thrombocytopenia, glucose level and aspartate aminotransferase were significant in IgM-only positive patients. Moreover, thrombocytopenia, leukopenia, glucose, aspartate aminotransferase and lymphopenia were independent predictors in both NS1 + IgM positive groups.

Conclusion: The study found that certain hematological and biochemical parameters can predict the outcome of dengue infection, which can assist physicians in the diagnosis and proper patient management. Parameters, such as thrombocytopenia, AST, hyperglycemia, and leukopenia with monocytosis (in the NS1-only phase); thrombocytopenia, elevated AST, and high blood glucose (in the IgM-only phase); and thrombocytopenia, elevated AST, high blood glucose, and leukopenia with lymphopenia (in the dual-positive/both NS1 + IgM phase), can provide insight into dengue positivity and help with patient management.

Keywords: dengue serology, RBC parameters

Introduction

Dengue virus belongs to the genus Flavivirus (group B arbovirus, RNA virus) and comprises structural and nonstructural proteins. [1] The classic clinical presentation is characterized by the abrupt onset of headache, myalgia and high fever, in addition to arthralgia, retro-orbital pain and hemorrhagic manifestations. The classical presentation differs from dengue hemorrhagic fever, which is characterized by fluid leakage into the interstitium. [2] Halstead states that in Latin American countries including Brazil, mosquito eradication was achieved in large urban centers after World War II, however, almost 20 years after, there was a resurgence of Aedes populations in Brazil. This allowed the first dengue outbreak with viral isolation in 1981 in Boa Vista, state of Roraima, along with the identification of DEN-1 virus and later of DEN-4 virus in 2007. [3] DEN-1 and DEN-2 was found in Rio de Janeiro in 1987 and DEN-3 in 2002. Between 2000 and 2005, Brazilian cases represented more than 60% of those registered by the World Health Organization (WHO) and almost 80% of all cases in the Americas. [4] This increase was identified in reports from the Brazilian Communicable Disease Information System. [5]

A quick dengue infection confirmation test is not available, and a complete blood count (CBC) might show characteristics such as leukopenia and thrombocytopenia. [6] These findings can be explained by peripheral platelet destruction mechanisms in the liver and spleen via the action of immunocomplexes or the complement system, in addition to the inhibition of medullary hematopoiesis [7] and disseminated intravascular coagulation. [8] A coagulogram might also indicate changes such as prolonged prothrombin and activated partial thromboplastin times, in addition to decreased serum fibrinogen concentration without increased levels of fibrinogen degradation products; these signs occur mainly with the hemorrhagic form of dengue fever. [9] According to Lupi, increased

aspartate aminotransferase (AST) can be observed in 30%-90% of classic dengue cases;8 this increase might correlate with thrombocytopenia, and thus, the Sri Lanka Medical Association considers a normal AST level to be a strong negative predictor of dengue hemorrhagic fever and its complications. [10]

arious diagnostic methods, such as virus-specific serological tests, molecular detection, and virus isolation, are used for the definitive diagnosis of DENV detection. [11] The three markers most commonly used in serological tests are NS1-Ag and IgM for acute infections and IgG for previous infections. NS1-Ag can be detected from the first 0–9 days of symptoms onset, while IgM is detected 4–5 days after symptoms onset, and production may continue approximately for 3 months or more post onset; IgG levels can be detected throughout the life, starting from 10 to 14 days of postinfection. [12,13] The gold standard test, like nucleic acid amplification tests, is not readily available in hospitals and clinics in a resource-limited country like Nepal. Thus, many of these facilities rely on lateral flow assays (LFA) or immunochromatography (ICT)-based detection methods for dengue diagnosis. Lateral flow assays are friendly to use and have a rapid turnaround time. Detection of NS1-Ag can be as sensitive as a molecular test during the first 0–7 days of onset of symptoms; however, detection can be compromised in secondary infection due to IgG antibodies from a previous infection. [13,14]

The aim of the present study was to assess the association of dengue serology with variations in RBC parameters.

Materials and Methods

The present study was conducted in the Department of Pathology, Darbhanga medical College and Hospital, Darbhanga, Bihar, India for one year. Out of 200 total study populations, 100 were dengue positive and 100 were dengue negative. After obtaining written informed consent, participants with fever/body pain along with a positive dengue profile test were included in the study. DENV-infected patients were categorized into NS1-only, IgM-only, and dual positive/both NS1 + IgM-positive groups. Study populations with negative dengue profile tests and abnormal hematological and biochemical profiles were excluded from the study. Patients with positive IgG in the dengue profile test were also excluded. Participants showing no symptoms and further tested negative for dengue profiles along with normal hematological and biochemical parameters were taken as a control group.

Specimen Collection and Processing

Following standard operating procedures, venous blood samples were collected. Whole blood was collected in a K3 EDTA vacuum tube and a gel and clot activator tube. A complete blood profile (hemoglobin, RBC and RBC indices, hematocrit, total leukocyte count, differential leukocyte count, and platelets) was performed from blood samples collected in a K3 EDTA tube with a hematology analyzer (Beckman Coulter DxH 520, USA). Similarly, a biochemistry analyzer (Selectra Pro S, ELITech Group, Netherlands) was used to perform biochemical analyses on enzymes (ALP, ALT, AST), bilirubin (total and direct), proteins (total protein and albumin), and nonprotein nitrogenous compounds (urea and creatinine) via a serum sample. Neutrophil: lymphocyte ratio (NLR), lymphocyte: monocyte ratio (LMR), and AST/ALT ratio were calculated based on data.

A serum sample was used to detect dengue infection. Qualitative dengue detection was based on the principle of the rapid chromatographic immunoassay (Dengue NS1 + IgM/IgG Combo Rapid Test, Healgen®). Patients with positive dengue cases were tested for either NS1 or IgM positivity or both NS1 and IgM positivity. Any result that was negative on any one of these profiles was treated as a dengue-negative case. All results were verified by a medical laboratory technologist and a microbiologist.

Statistical Analysis

The data were analyzed using IBM SPSS version 25. Shapiro–Wilk normality test was applied to analyze the data for normal distribution. Categorical variable were described as in numbers and percentage. Continuous variables were shown as the median (Q3−Q1). Univariate analysis was performed appropriately using the Mann–Whitney U test, which was used for overall analysis between dengue positive and dengue negative groups. Likewise, Kruskal Wallis H test, an omnibus test statistic was used to compare > 2 groups. Furthermore, in the case of statistical association, pairwise analysis was performed via Dunn's post hoc test with Bonferroni adjustment, and a p value <0.05 was considered significant. Parameters that were mutually significant in both the univariate analysis and the comparative analysis were included in a univariate logistic regression analysis where a p value <0.25 was considered significant. In the multivariate logistic regression, a few parameters were added despite insignificant results in univariate logistics due to their clinical relevance. Binary logistic regression (in a dichotomous outcome) and multinomial logistic regression (more than 2 outcomes) were performed as required. Results were presented as crude and adjusted odds ratios with a 95% confidence interval (95% CI). Those variables that yielded the lowest p value <0.05 in multivariate

logistics have been considered statistically significant.

Results

Table 1: Hematological and biochemical findings in study participants

	Dengue negative	Dengue positive	P Value
	Median (Q_3–Q_1)	Median (Q_3–Q_1)	
Age (years)	32.0 (45.0–21.0)	36.0 (55.0–24.0)	0.006
Hemoglobin (gm/dl)	13.8 (15.03–13.35)	14.4 (15.67–12.89)	0.673
RBC (X 10^{12}/L)	4.66 (5.01–4.31)	4.78 (5.19–4.43)	0.983
HCT (%)	40.0 (43.5–37.9)	40.7 (44.15–36.65)	0.863
MCV (fl)	86.0 (89.0–83.0)	84.9 (88.25–81.3)	0.048
MCH (pg)	30.2 (31–28.8)	30.0 (82.55–31.30)	0.965
MCHC (gm/dl)	35 (35.2–34.0)	35.5 (36–35.2)	<0.001
TLC (cells per μl)	6600 (7500–5370)	3885 (5790–2950)	<0.001
Neutrophil (%)	64 (69–58)	67 (76–59)	0.854
Lymphocyte (%)	27 (32–23)	21 (30–14)	0.765
Monocyte (%)	7 (8–5)	9 (13–7)	<0.001
Eosinophil (%)	2 (3–1)	1 (2–1)	<0.001
Platelets (per μl)	286000 (336000–223000)	164000 (192000–127000)	<0.001
NLR	2.37 (2.91–1.8)	3.37 (5.61–1.96)	0/789
LMR	4.29 (5.5–3.5)	2.57 (3.78–1.46)	<0.001
Glucose (mg/dl)	87.66 (111.24–73.08)	98.1 (117–86.0)	<0.001
Urea (mg/dl)	23.34 (31.26–20.74)	21.78 (27.24–17.1)	0.998
Creatinine (mg/dl)	0.84 (0.95–0.73)	0.92 (1.17–0.76)	0.964
Total bilirubin (mg/dl)	0.48 (0.58–0.45)	0.50 (0.65–0.40)	0.009
Direct bilirubin (mg/dl)	0.11 (0.14–0.05)	0.10 (0.12–0.86)	0.678
Total protein (gm/dl)	6.9 (7.3–6.4)	7.06 (7.38–6.65)	0.006
Albumin (gm/dl)	4.2 (4.8–3.9)	4.03 (4.32–3.7)	<0.001
ALP (U/L)	145 (169–105)	73.77 (129.6–61.07)	<0.001
AST (U/L)	25 (28–21)	37.51 (56.23–28.06)	<0.001
ALT (U/L)	29 (38–24)	30.64 (48.7–17.47)	<0.001
AST/ALT ratio	0.82 (0.93–0.72)	1.29 (1.73–1.02)	<0.001

The overall association of laboratory findings between the dengue positive and negative groups was presented in the table 1.

Table 2: Comparison of hematological and biochemical findings in NS1, IgM, and NS1 + IgM positive dengue patients

Parameters	**Dengue positive**		
	NS1 only	**Both NS1 + IgM**	**IgM only**
	Median (Q3–Q1)	**Median (Q3–Q1)**	**Median (Q3–Q1)**
Hemoglobin (gm/dl)	14.64 (15.87–12.90)	14.34 (15.90–12.97)	14.2 (14.7–12.62)
RBC (X 10^{12}/L)	4.78 (5.3–4.24)	4.74 (5.19–4.49)	4.84 (5.09–4.42)
HCT (%)	41.5 (44.9–35.9)	40.5 (45.42–37.7)	40.66 (42.5–36.25)
MCV (fl)	86.4 (88.8–83.3)	84.6 (88.12–80.2)	83.7 (87.25–79.7)
MCH (pg)	30.8 (31.8–29.7)	30.0 (31.32–28.2)	29.24 (30.0–27.52)
MCHC (gm/dl)	35.5 (36–35.2)	35.2 (35.82–3.5)	34.75 (35.5–33.9)
TLC (cells per μl)	3880 (5790–2950)	3345 (4442–2497)	4800 (6525–3072)
Neutrophil (%)	67 (76–59)	56.5 (67.75–46)	55 (69–44.25)
Lymphocyte (%)	21 (30–14)	33 (40–22)	32.5 (46–20.25)
Monocyte (%)	9 (13–7)	9.5 (12.25–6)	7 (10.75–5.0)
Eosinophil (%)	1 (2–1)	2 (3–1)	1 (2–1)
Platelets (per μl)	164000 (192000–127000)	113000 (153500–87000)	131000 (177500–53500)
NLR	3.36 (5.61–1.97)	1.83 (3.04–1.12)	1.75 (3.5–1.00)
LMR	2.57 (3.77–1.46)	3.25 (4.23–2.57)	3.9 (6.12–2.34)
Glucose (mg/dl)	98.1 (117–86.0)	104.5 (144.9–91.0)	122 (173–90.9)

Urea (mg/dl)	21.78 (27.24–17.1)	22.13 (31–17.64)	30.04 (37.05–22.75)
Creatinine (mg/dl)	0.92 (1.17–0.76)	0.82 (0.97–0.64)	0.90 (0.99–0.72)
Total bilirubin (mg/dl)	0.49 (0.64–0.4)	0.52 (0.7–0.4)	0.51 (0.66–0.4)
Direct bilirubin (mg/dl)	0.1 (0.12–0.865)	0.1 (0.2–0.08)	0.1 (0.15–0.865)
Total protein (gm/dl)	7.06 (7.38–6.65)	7.24 (7.56–6.89)	6.95 (7.9–6.55)
Albumin (gm/dl)	4.03 (4.31–3.7)	4.1 (4.31–3.86)	4.1 (4.31–3.66)
ALP (U/L)	73.77 (129.6–61.07)	106.4 (207.3–69.28)	139.5 (192.5–90.72)
AST (U/L)	37.51 (56.23–28.06)	76.27 (119.9–44.46)	113 (197–36.75)
ALT (U/L)	30.64 (48.7–17.47)	62.21 (98.9–36.0)	92.42 (156.75–43.5)
ST/ALT ratio	1.29 (1.73–1.02)	1.23 (1.58–1.06)	1.17 (1.48–0.95)

Multiple logistic regression showed thrombocytopenia, leukopenia (ORA = 0.999; $p < 0.001$), glucose level, aspartate aminotransferase and monocytosis as significant parameters in the NS1-only positive group. Similarly, thrombocytopenia, glucose level and aspartate aminotransferase were significant in IgM-only positive patients. Moreover, thrombocytopenia, leukopenia, glucose, aspartate aminotransferase and lymphopenia were independent predictors in both NS1 + IgM positive groups.

Discussion

Dengue virus (DENV) is a 50 nm, single-stranded RNA virus with a genome approximately 11 kb in length. [15] The virus contains three structural genes encoding capsid protein (C), membrane protein (M), and envelope protein (E), as well as seven nonstructural (NS) genes encoding NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 proteins. [16] Dengue virus is transmitted primarily by the vectors Aedes aegypti and Aedes albopictus and is most prevalent in tropical and subtropical areas. Dengue infection is usually asymptomatic and self-curable. [17] The World Health Organization (WHO) classified symptomatic dengue as dengue with or without warning signs and severe dengue. [18] The incubation period of the virus ranges from 3 to 10 days, typically 5–7 days, and follows a clinical course as a biphasic febrile phase lasting 2–7 days, a critical phase which lasts 24–48 hours, and a convalescent phase. [19] With an estimated infection of about 400 million people annually, the disease now affects more than 100 countries, most of them in Asia, with a disease burden of 70%. [17]

Multiple logistic regression showed thrombocytopenia, leukopenia (ORA = 0.999; $p < 0.001$), glucose level, aspartate aminotransferase and monocytosis as significant parameters in the NS1-only positive group. Similarly, thrombocytopenia, glucose level and aspartate aminotransferase were significant in IgM-only positive patients. Moreover, thrombocytopenia, leukopenia, glucose, aspartate aminotransferase and lymphopenia were independent predictors in both NS1 + IgM positive groups. As per the WHO, hematocrit and thrombocytopenia are the most important laboratory parameters measured during dengue infection. [20] But, our study showed no significant association between hematocrit during the serological course of NS1 and IgM. Few studies report similar findings of insignificance; this may be because our study included only dengue fever patients with mild primary active infections. Thus, there is less chance of plasma leakage, which does not indicate abnormal hematocrit results. [21,22] Thrombocytopenia, which is well correlated with dengue severity as shown by various studies, also remained significant in our study. [23-26] This decrease in platelets may be due to low production or increased destruction of platelets via activation of the complement factor C3 and further binding of the C5b-9 complex to the platelet surface. [27]

In our study, the median increase in ALT, AST, and ALP was significantly associated with the dengue-positive group; nonetheless, only AST was found to be independently associated. This finding corresponds to studies supporting higher transaminase levels during dengue positivity. [28,29] ALT is primarily of hepatic origin, while AST is of both hepatic and nonhepatic origin; hence, damage to nonhepatic tissues can also elevate AST as compared to ALT. [30] As a result, despite the significant results obtained in this study, a higher level of AST may not correctly represent hepatic involvement in the dengue-positive group. [31] Furthermore, the recommended drug to minimize dengue symptoms is acetaminophen, which even at therapeutic dosage can cause a temporary elevation in transaminase levels. [32,33]

Conclusion

The study found that certain hematological and biochemical parameters can predict the outcome of dengue infection, which can assist physicians in the diagnosis and proper patient management. Parameters, such as thrombocytopenia, AST, hyperglycemia, and leukopenia with monocytosis (in the NS1-only phase); thrombocytopenia, elevated AST, and high blood glucose (in the IgM-only phase); and thrombocytopenia, elevated AST, high blood glucose, and leukopenia with lymphopenia (in the dual-positive/both NS1 + IgM phase), can provide insight into dengue positivity and help with patient management.

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