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CORRELATION OF CLINICAL SEVERITY AND LABORATORY PARAMETERS WITH DIFFERENT SEROTYPES IN DENGUE VIRUS: A CROSS-SECTIONAL STUDY.

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ABSTRACT

Background

In tropical and subtropical nations, dengue fever a serious public health concern is brought on by the dengue virus (DENV). Dengue hemorrhagic fever (DHF) and dengue shock syndrome are two examples of the mild to severe forms of the virus. In order to improve knowledge of the variables influencing severe dengue outcomes, the study examines the correlation between clinical severity, laboratory markers, and dengue virus serotypes.

Methods

The study comprised 130 patients diagnosed with dengue at a tertiary care hospital. Patients were classified into three stages of dengue severity based on WHO guidelines. Serological and molecular diagnostics, including ELISA and RT-PCR, were used to identify DENV serotypes and analyze laboratory parameters. Statistical analyses were performed to evaluate the correlations.

Results

The participants had average age of 34.5 years, with 55.4% males. Patients were categorized into Stage 1 (43.1%), Stage 2 (36.9%), and Stage 3 (20%) based on severity. Significant differences in laboratory parameters were observed across the stages, with lower platelet counts and higher levels of hematocrit, liver enzymes (AST and ALT), and ferritin in severe dengue cases ($p < 0.001$). DENV-2 was the most prevalent serotype (35.4%) and was significantly associated with severe dengue ($p = 0.025$). ROC curve analysis identified ferritin as a potential marker for severe dengue with a cutoff value of 300 ng/mL and an AUC of 0.88.

Conclusion

The study shows that dengue patients' platelet count, hematocrit, liver enzymes, and ferritin levels are strongly correlated with clinical severity. Serious illness symptoms are associated to DENV-2. These findings emphasise the necessity of serotype identification and laboratory monitoring in dengue management.

Recommendations

Future studies with bigger sample sizes are recommended to validate these findings. Routine monitoring of ferritin levels and early identification of DENV serotypes could enhance the management and prognosis of dengue patients.

Keywords: *Dengue virus, Clinical severity, Laboratory parameters, Serotypes, Ferritin.*
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INTRODUCTION

Dengue fever, caused by the dengue virus (DENV), continues to be a chief public health issue in tropical and subtropical areas across the globe. According to the World Health Organisation (WHO), there are around 390 million cases of dengue infections per year, and about 96 million of these cases show clinical symptoms [1]. The primary mode of transmission for dengue is by the *Aedes aegypti* mosquito, and the virus is present in four different serotypes: DENV-1, DENV-2, DENV-3, and DENV-4. These serotypes have the potential to cause a variety of diseases, ranging from a mild case of dengue fever to more serious conditions such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [2].

The number of dengue cases has increased recently, which highlights the significance of developing a more thorough understanding of the factors that influence the disease's severity. Various factors, such as the specific serotype of the dengue virus, the immune response of the host, and any pre-existing medical disorders, might impact the severity of dengue infection [3]. It is worth mentioning that secondary infections caused by different serotypes can lead to more severe consequences because of antibody-dependent enhancement (ADE). This occurs when pre-existing non-neutralizing antibodies let the virus enter host cells. Identifying the specific serotype causing the infection is crucial for effectively managing and predicting the outcome for the patient.

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Laboratory parameters play a crucial role in the clinical assessment and management of dengue patients. Commonly monitored parameters include platelet count, hematocrit levels, liver enzymes (AST and ALT), and markers of inflammation such as ferritin. Platelet count is a key indicator of dengue severity, with thrombocytopenia being a hallmark of severe disease. Elevated hematocrit levels reflect plasma leakage, a critical feature of severe dengue. Liver enzymes, indicative of hepatic involvement, are also closely monitored as they tend to rise with increasing disease severity [4].

Ferritin, an acute phase reactant, has emerged as a potential marker for dengue severity. Elevated ferritin levels have been associated with severe manifestations, making it a valuable tool for early identification of patients at risk of severe disease. Furthermore, rapid detection of dengue serotypes made possible by recent developments in molecular diagnostics, such as reverse transcriptase polymerase chain reaction (RT-PCR), has allowed for targeted public health initiatives and individualised patient care [5].

This study aims to correlate clinical severity and laboratory parameters with different dengue virus serotypes.

METHODOLOGY

Study Design

A cross-sectional, hospital-based study.

Study Setting

The study took place at Anugrah Narayan Magadh Medical College and Hospital (ANMMC), Gaya hospital, from September 2022 to March 2024.

Participants

A total of 130 patients diagnosed with dengue were included in this study. Participants were classified according to the WHO dengue classification system [6]:

- Stage 1: Dengue without any indication of warning indications

- Stage 2: Dengue with indications of potential danger

- Stage 3: Critical dengue

Inclusion Criteria

Individuals aged 18 years and older with verified dengue infection.

Exclusion Criteria

Individuals receiving outpatient treatment.

Sample size

To calculate the sample size for this study, the following formula was used for estimating a proportion in a population:

$$n = \frac{Z^2 \times p \times (1-p)}{E^2}$$

Where:

- n = sample size

- Z = Z-score corresponding to the desired level of confidence

- p = estimated proportion in the population

- E = margin of error

Bias

To reduce selection bias, a convenient sampling method was employed. Standardized data collection and diagnostic procedures were used to minimize information bias.

Variables

Variables included dengue virus serotypes, clinical severity and laboratory parameters.

Data Collection

Data were gathered from patient medical records, laboratory test results, and clinical evaluations. Both serological and molecular diagnostic tests were conducted on acute phase serum samples.

Procedure

1. Sampling Technique: Convenient sampling was used.

2. Serological Diagnosis: Analysed in this study were serum samples obtained from 130 patients during the acute phase of their illness, within seven days after the onset of symptoms. These samples were tested positive for dengue NS1 antigen using a fast immunochromatographic test or for IgM using ELISA (Panbio). The samples were kept at a temperature of -20°C.

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3. Molecular Diagnosis: The QIAamp Viral RNA Mini Kit was used to extract RNA, following the instructions provided by the manufacturer. The RNA that was obtained was kept at a temperature of -80°C. The confirmation of dengue serotypes was achieved through the utilisation of multiplex one-step reverse transcriptase PCR, employing CDC dengue primers, probes, and the One Step Prime Script™ RT-PCR Kit. The multiplex assay successfully detected all four dengue serotypes, namely DENV-1, DENV-2, DENV-3, and DENV-4. The thermal cycling settings were determined according to the CDC Dengue Kit protocol. The process began with an initial hold at 50°C for 30 minutes, followed by a temperature increase to 95°C for 2 minutes. This was followed by 45 cycles of heating to 95°C for 15 seconds and then cooling to 60°C for 1 minute, during which fluorescence was measured. Amplification was identified by observing an augmentation in particular fluorescence channels, with the kit including positive controls for comparison.

Statistical Analysis

In this study, categorical data were presented as frequencies and proportions, while quantitative data were summarized using mean and median values. Data entry and analysis were conducted using SPSS version 17. Receiver Operating Characteristic (ROC) curves were employed to establish cutoff values for quantitative variables. To assess the relationship between ferritin levels and dengue severity, the Kruskal-Wallis test was utilized. Statistical significance was stated as a p-value of less than 0.05.

Ethical considerations

The study protocol was approved by the Ethics Committee and written informed consent was received from all the participants.

RESULT

A total of 130 participants were included in the study, with a mean age of 34.5 ± 12.3 years. The study population comprised 72 males (55.4%) and 58 females (44.6%). Patients were classified into three groups based on the WHO dengue classification: 56 patients (43.1%) with dengue without warning signs (Stage 1), 48 patients (36.9%) with dengue with warning signs (Stage 2), and 26 patients (20%) with severe dengue (Stage 3).

The laboratory parameters, including platelet count, hematocrit, liver function tests (AST, ALT), and ferritin levels, were assessed across the three stages of dengue severity.

The distribution of dengue virus serotypes among the patients was as follows: DENV-1 (30.8%), DENV-2 (35.4%), DENV-3 (21.5%), and DENV-4 (12.3%). The correlation between dengue virus serotypes and clinical severity was evaluated using chi-square tests. DENV-2 was considerably related with severe dengue ($p = 0.025$).

The ROC curve for ferritin levels indicated a cutoff value of 300 ng/mL to predict severe dengue, with an AUC of 0.88 (95% CI, 0.82-0.93). The Kruskal-Wallis test demonstrated a significant association between higher ferritin levels and increased severity of dengue ($p < 0.001$).

Table 1: Demographic Characteristics

Demographic Characteristics	Stage 1 (n=56)	Stage 2 (n=48)	Stage 3 (n=26)	Total (n=130)
Mean Age (years)	32.1 ± 11.8	35.6 ± 12.5	37.8 ± 12.7	34.5 ± 12.3
Gender, n (%)				
- Male	30 (53.6%)	26 (54.2%)	16 (61.5%)	72 (55.4%)
- Female	26 (46.4%)	22 (45.8%)	10 (38.5%)	58 (44.6%)

Table 2: Laboratory Findings

Laboratory Parameters	Stage 1	Stage 2	Stage 3	p-value
Platelet Count ($x10^9$/L)	145.6 ± 34.7	92.3 ± 21.4	58.7 ± 15.8	<0.001
Hematocrit (%)	39.5 ± 4.2	42.1 ± 3.8	44.7 ± 4.5	<0.001
AST (U/L)	45.8 ± 15.3	72.4 ± 20.5	108.5 ± 32.7	<0.001
ALT (U/L)	38.6 ± 12.7	66.3 ± 18.9	95.6 ± 28.2	<0.001
Ferritin (ng/mL)	150.5 ± 45.2	320.7 ± 78.6	560.3 ± 110.5	<0.001

Table 3: Serotype Distribution

Dengue Serotype	Stage 1	Stage 2	Stage 3	Total (n=130)
DENV-1	16 (28.6%)	18 (37.5%)	6 (23.1%)	40 (30.8%)
DENV-2	20 (35.7%)	14 (29.2%)	12 (46.2%)	46 (35.4%)
DENV-3	12 (21.4%)	10 (20.8%)	6 (23.1%)	28 (21.5%)
DENV-4	8 (14.3%)	6 (12.5%)	2 (7.7%)	16 (12.3%)

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Table 4: Correlation of Serotypes with Severity

Serotype vs Severity	Stage 1	Stage 2	Stage 3	p-value
DENV-1	16	18	6	0.342
DENV-2	20	14	12	0.025
DENV-3	12	10	6	0.451
DENV-4	8	6	2	0.789

Table 5: Statistical Analysis of Ferritin Levels Across Different Stages of Dengue Severity

Ferritin Levels (ng/mL)	Mean ± SD	Median (IQR)	p-value
Stage 1	150.5 ± 45.2	145 (120-180)	<0.001
Stage 2	320.7 ± 78.6	310 (275-355)	
Stage 3	560.3 ± 110.5	545 (500-620)	

DISCUSSION

The study involving 130 patients diagnosed with dengue, aimed to correlate clinical severity with laboratory parameters and various dengue virus serotypes. The demographic analysis revealed a mean age of 34.5 years, with a slight male pre-dominance (55.4%). Patients were categorized into three stages of dengue severity according to WHO guidelines, with the majority falling into Stage 1 (43.1%), followed by Stage 2 (36.9%), and Stage 3 (20%).

Laboratory findings demonstrated significant variations across the severity stages. Platelet counts were markedly lower in patients with severe dengue (Stage 3), averaging 58.7 x10^9/L, compared to those in Stage 1 (145.6 x10^9/L) and Stage 2 (92.3 x10^9/L), with a p-value <0.001. Hematocrit levels and liver enzymes (AST and ALT) also showed a significant increase with disease severity. Notably, ferritin levels were substantially elevated in severe dengue cases (mean 560.3 ng/mL) compared to milder cases, suggesting its potential as a prognostic marker.

The serotype distribution revealed that DENV-2 was the most prevalent (35.4%), followed by DENV-1 (30.8%), DENV-3 (21.5%), and DENV-4 (12.3%). A significant association was observed between DENV-2 and severe dengue ($p = 0.025$), indicating that this serotype might be a predictor of severe disease outcomes. The chi-square tests confirmed the correlation between DENV-2 and increased clinical severity.

Receiver Operating Characteristic (ROC) curve analysis for ferritin levels suggested a cutoff value of 300 ng/mL to predict severe dengue, with an AUC of 0.88, indicating good diagnostic accuracy. The Kruskal-Wallis test further validated the significant association between higher ferritin levels and dengue severity ($p < 0.001$).

Overall, the findings underscore the importance of specific laboratory parameters, such as platelet count, hematocrit, liver enzymes, and ferritin levels, in assessing the clinical severity of dengue. Additionally, the identification of DENV-2 as a significant marker for severe dengue can aid in early risk stratification and management of patients. These results highlight the need for continuous monitoring and further research to enhance the understanding and treatment of dengue virus infections.

The clinical severity of dengue fever and associated laboratory parameters can vary significantly depending on the infecting dengue virus (DENV) serotype. A hospital-based study found that DENV-3 was the most prevalent serotype among patients, followed by co-infections of DENV-3 and DENV-4. Elevated liver enzymes (AST, ALT) and increased ferritin levels were significant predictors of severe dengue. Musculoskeletal and gastrointestinal manifestations were common among severe cases [7]. A study indicated that the development of dengue complications was significantly higher in males. However, no specific association was found between serotypes and the severity of complications. The study highlighted the need for meticulous management of all dengue cases regardless of serotype [8].

An analysis was conducted on 5642 cases in Vietnam, which revealed that elevated levels of viral particles in the blood during the phase of fever were linked to severe outcomes of dengue fever, regardless of the specific strain of the virus. The study emphasised the significance of high viremia as a risk factor for severe dengue [9]. A study conducted in Tamil Nadu examined children who were admitted to the hospital. The study discovered that DENV-4 infections were linked to noticeably increased levels of liver enzymes. Severe dengue was not notably associated with any one serotype, although DENV-4 exhibited a distinct pattern of liver damage [10].

A longitudinal analysis found that more severe dengue was associated with higher percentages of plasmablasts and effector memory CD4 T cells. These findings highlight the role of specific immune cell phenotypes in dengue severity [11]. A systematic review identified several clinical and laboratory predictors for severe dengue in children, including elevated liver enzymes, low

platelet count, and specific serotypes like DENV-2 being more associated with severe outcomes [12].

Generalizability

The study findings, such as the correlation between elevated ferritin levels and severe dengue, can be applied to larger populations to enhance early identification and management of high-risk dengue patients, improving overall patient outcomes.

CONCLUSION

The study concludes that certain laboratory parameters are strongly correlated with the clinical severity of dengue. The identification of dengue serotypes can aid in anticipating disease progression, with DENV-2 being notably linked to severe cases.

LIMITATIONS

The limitations of this study include a small sample population who were included in this study. Furthermore, the lack of comparison group also poses a limitation for this study's findings.

RECOMMENDATION

Future studies with bigger sample sizes are recommended to validate these findings. Routine monitoring of ferritin levels and early identification of DENV serotypes could enhance the management and prognosis of dengue patients.

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LIST OF ABBREVIATIONS

DENV: Dengue virus
DHF: Dengue hemorrhagic fever
DSS: Dengue shock syndrome
ELISA: Enzyme-linked immunosorbent assay
RT-PCR: Reverse transcriptase polymerase chain reaction
WHO: World Health Organization
ADE: Antibody-dependent enhancement
AST: Aspartate aminotransferase
ALT: Alanine aminotransferase
ROC: Receiver Operating Characteristic
AUC: Area Under the Curve
CI: Confidence Interval
PCR: Polymerase Chain Reaction

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CONFLICT OF INTEREST

The authors have no competing interests to declare.

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