Original Article

Predicting the Unpredictable: Hematological and Biochemical Parameters as Predictive Markers of Dengue Severity in Patients without Warning Sign

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ABSTRACT

Objective: To evaluate the biochemical and hematological alterations in patients having Dengue without Warning Signs (DWWS) for predicting the severity of disease

Study Design: Observational Case Control study

Place and Duration of Study: This study was conducted at the Clinical Pathology Department of Jinnah Post Graduate Medical Center (JPMC), Karachi, from November 2022 to February 2023.

Materials and Methods: The study encompassed 200 participants, presented at the medical and surgical OPDs, suspected for DWWS. These fulfilled the inclusion criteria (acute febrile illness, generalized rash on the body, vomiting, retro-orbital pain, and arthralgia/myalgia. Febrile illness regarded as body temperature above 38.40 Fahrenheit). On the basis of positive NS 1 or IgM or both patients were assigned to DWWS group (100 individuals) where as those with negative test results, to the control group. Blood samples were collected, from both groups, for the assessment of hematological and biochemical parameters. The data organization and analysis was performed using SPSS software version 20.

Results: Out of the 200 participants with febrile presentation, those positive for DENV exhibited an elevated mean hematocrit (49.06±3.89) and a low platelet count (73.64 ±28.86). Biochemical alterations in the DWWS group included significantly elevated levels of mean GGT (49. ± 49.42), Serum ferritin (251.79 ±472.79) , hsCRP (11.73±20.39) & serum triglycerides (230.60±275.51), where serum cholesterol (124.30±59.85), Vitamin D levels (14.241±7.11) were significantly low in this group compared to the controls.

Conclusion: This study indicated the potential role of different biochemical and hematological parameters as Predictive markers for severity of dengue.

Key Words: Dengue Without Warning Signs , Vitamin D, Ferritin, Hematocrit, Thrombocytopenia

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INTRODUCTION

Due to its high morbidity and mortality rate, Dengue is considered one of the most critical and widespread arthropod borne viral disease affecting humans. It can be caused by any of the 4 distinct serotypes of Dengue Virus (DENV) and is transmitted primarily by Aedius aegypti mosquito bite[6]. Dengue affects approximately 100 million individuals in endemic regions every year[1,2]. The mortality rate for dengue globally is really low, but it may rise up to 20% without prompt care[3]. Clinical manifestations of DENV infection can vary widely, ranging from asymptomatic to patients exhibiting a variety of symptoms. WHO has classified symptomatic DENV infections into 2 main groups on the basis of types and severity of symptoms; Dengue Fever (with/without warning signs) and Severe Dengue (SD). During the initial phase, the disease is termed Dengue Fever (DF) and is characterized by fever accompanied by ≥2 symptoms of the following: body ache, nausea, vomiting, rash, retro-orbital pain, leucopenia[7]. Some patients may develop potentially fatal Severe Dengue (SD) indicated by plasma leakage, hemorrhaging and shock.

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Warning signs indicating progression towards several dengue include: hepatomegaly, hematemesis, hematochezia, fatigue, vomiting persistently, fluid accumulation in pleural space &/or abdomen, restlessness, buccal mucosal bleeding[4, 5.]

Due to delayed presentation of warning signs and lack of specific antiviral therapy, SD dengue may result in fatal outcomes. Only timely diagnosis and proper management can lower the mortality rate and disease severity[5, 12]. Identification of biomarkers which help in proper diagnosis & risk factors, that help envision the severity of disease in the early stage of infection are the need of the hour[5]. Serological tests like detecting dengue NS1 Antigen or the dengue IgM antibody using ELISA, are the diagnostic tools for definite diagnosis. But they are expensive & unavailable in many hospitals[15].

Alterations in hematological parameters is observed during the course of infection, including initial mild leukocytosis followed by progressive leucopenia, thrombocytopenia& an increased hematocrit; indicator of vascular leakage and atypical lymphocytosis. These dynamic variables can be used for diagnosis &assessment clinical outcomes, early in the infection[11,12]. Additionally many biochemical changes can be utilized as a Prognostic biomarker for the DENV infection. These include change in concentration of liver enzymes and serum albumin.

Studies have claimed that hyperferritinemia can be used as a predictive biomarker for development of severe dengue[7,8,9]. Increased ferritin levels are an indicator of active disease state, characterized by an overly active immune system and failure of coagulation mechanisms.

Nutritional status of the patient could also be an indicator of the severe outcomes of the infection. Other than its role in bone health, vitamin D also functions as an immunomodulator, having a role in macrophage maturation and phagocytosis, and aiding in the synthesis of pro-inflammatory cytokines and cell-mediated immunity[13]. Many studies have investigated the correlation between vitamin D levels and dengue fever, with results indicating the association between vitamin D deficiency and progression towards severe dengue[14].

This study aimed to assess the above mentioned biochemical and hematological parameters in patients having dengue without warning signs (DWWS) to predict the severity of disease.

MATERIALS AND METHODS

This observational case control study was conducted at the Clinical Pathology Department of Jinnah Post Graduate Medical Center (JPMC), Karachi, during November 2022 to February 2023, throughout the recent dengue outbreak in the city. Our study population comprised of 200 individuals presented at Medical and surgical OPDs with suspect of Dengue without Warning Signs. The inclusion criteria were patients with acute febrile illness, generalized rash on the body, vomiting, retro-orbital pain, and arthralgia/myalgia. Febrile illness regarded as body temperature above 38.40 Fahrenheit suspected to acquire dengue virus infection. Febrile patients presented to OPD with other diagnosed cases of typhoid, malaria, and chronic disorders like tuberculosis were excluded from the study population.

Pre-designed questionnaire was used to collect the information regarding baseline characteristics of all study participants.

Samples were assessed for Dengue virus by performing Immunochromatographic assays. To summarize the diagnostic protocol employed in the analysis included IgM enzyme-linked immunosorbent assay (ELISA) in matched sera (Dengue NS1 Rapid Test Cassette- Citest) and NS1 antigen detection (Dengue NS1 Rapid Test Cassette- Citest), for confirmation of DENV infection.

Based on the results patients with acute febrile illness ,generalized rash on the body, vomiting, retro-orbital pain ,arthralgia/myalgias, amid non-structural protein-1 (NS 1) antigen or Immunoglobulin M (Ig M antibody) detected positive were categorized as cases of dengue without warning signs whereas equal number of individuals with febrile presentation but NS1 antigen, or Ig M antibody found negative were considered as control group.

For assessment of biochemical variables 5 ml of whole blood was collected in lithium heparin containing vaccutainers (green top). The samples were centrifuged and serum was separated for assessment of biochemical variables. Serum Ferritin and Vitamin D levels were measured through a chemiluminescence assay on LIAISON® XS – DiaSorin automated immunoassays analyzer. Valuation of Lipid profile (Serum triglycerides, serum cholesterol, serum LDL & serum HDL), Liver Enzymes (Alanine aminotransferases, Aspartate aminotransferases) & hs C reactive protein (hsCRP) was conducted on an automated clinical chemistry analyzer, (Beckman coulter AU 5800), half an hour after sample collection.

Whereas 5 ml of samples were collected in EDTA containing vaccutainer(purple top) for investigation of hematological variables (total leucocyte count, differential leucocyte count , hemoglobin, platelet count and hematocrit).These samples were mixed thoroughly on roller mixer and analyzed within half hour of collection using SYSMEX XN 1000™ Hematology Analyzer.

Reference ranges applied in JPMC clinical lab were referred to draw cutoff values of each hematological and biochemical parameter

Data analysis was done using the Statistical package for the Social Sciences (SPSS) Version 20. Continuous variables were summarized using mean and standard deviation (SD) and categorical variables as frequencies

and percentages. A two tailed probability value of <0.05 (95% CI) was accepted at the level of statistical significance. Independent sample t test and paired sample t test were applied to compare the biochemical and hematological parameters between cases and control groups respectively.

RESULTS

This study encompassed a total of 200 individuals, which were recruited based on presentation of febrile illness. These were categorized into two groups on the basis of serological confirmation for dengue. Patients with positive IgM, NS1, IgG, or two of these or all three, were assigned to the Dengue without warning signs group where as those with negative serologic results were assigned to the control group. Baseline characteristics of both groups are mentioned in Table 1.
Infection was more prevalent in the younger age groups (26-45 years) and the female gender. A higher incidence of fever, myalgias, arthralgias and retro-orbital pain was observed in the dengue fever group. Table No.1: Baseline Characteristics of dengue and other Febrile illness.
The results from the hematological profile (Table No.2), revealed a higher mean hematocrit value (49.06%, ± 3.89) and a significantly lower mean platelet count (73.64±28.86), absolute neutrophil count, lymphocyte count compared to the control group.

Moreover dengue group displayed elevated levels of mean serum ferritin (251.79 ±472.79) and hsCRP (11.73±20.39), indicating increased inflammation (Table No. 3).
Major alterations in the lipid panel (Table 4) of the infected group were higher levels of mean serum triglycerides (230.60±275.51) and lower levels of serum cholesterol (124.30±59.85), serum LDL (132.24±31.66), and serum HDL (17.68 ±19.18).
A notable finding in the liver profile (Table 5) of the dengue group was significantly high GGT levels. Additionally vitamin D deficiency (VDD) was found in the dengue group.

Table No. 1: Baseline Characteristics of the patients

Clinical features	Dengue fever	Other Febrile illness
Fever	70	65
Myalgias	44	26
Arthralgias	58	31
Vomiting	07	11
Retro orbital pain	51	00
Rashes	10	00
Jaundice	00	18
Age distribution of the patients		
18 to 25 years	19	14
26 to 35 years	41	25
36 to 45 years	28	30
46 to 55 years	06	11
>55 years	12	20
Gender distribution of the patients		
Males	43	67
Females	57	33

Table No. 2: Comparative analysis of hematological parameters in dengue and other febrile illness.

Parameters	Dengue Mean (Standard deviation)	Febrile illness other than Dengue Mean (Standard deviation)	P value
Hemoglobin(g/dl)	15.76 (2.02)	12.99 (1.26)	0.001*
Hematocrit (%)	49.06 (3.89)	39.73 (3.82)	0.001*
Total leukocyte count /cumm	3.068 (1.006)	3.301 (1.123)	0.123*
Platelet count /cumm	73.64 (28.86)	03.70 3 (423.50)	0.001*
Absolute neutrophil count	4.96 (1.90)	6.14(1.14)	0.001*
Absolute lymphocyte count	1.72 (0.59)	3.30 (0.47)	0.001*

Table No. 3: Comparative analysis of ancillary biochemical parameters between dengue and other febrile illness

Parameter	Dengue Mean (Standard deviation)	Febrile illness other than Dengue Mean (Standard deviation)	P value
hs CRP	11.73(20.39)	7.06 (12.18)	0.51
Serum Vitamin D	14.241 (7.11)	26.88 (21.05)	0.001
Serum Ferritin	251.79 (472.79)	73.97 (104.81)	0.01

Table No.4: Comparative analysis of Lipid profile of dengue and other febrile illness

Parameters	Dengue Mean (Standard deviation)	Febrile illness other than Dengue Mean (Standard deviation)	P value
Serum Triglyceride	230.60 (275.51)	117.78 (46.81)	0.001*
Serum Cholesterol	124.30 (59.85)	167.06(38.63)	0.001*
Serum LDL	60.930(25.53)	132.24 (31.66)	0.001*
Serum HDL	17.68(19.18)	56.115(44.85)	0.001*

Table No. 5: Comparative analysis of Liver Profile of dengue and other febrile illness

Parameter	Dengue Mean (Standard deviation)	Febrile illness other than Dengue Mean (Standard deviation)	P value
Serum GGT	49. (49.42)	28.50 928.85)	0.001*
Serum AST	28.60 (31.89)	30.20 (33.62)	0.730
Serum ALT	82.75 (44.56)	85.200 (23.77)	0.630
Total bilirubin	0.497 (0.375)	0.473(0.195)	0.580
Direct bilirubin	0.215 (0.198)	0.250 (0.145)	0.154

DISCUSSION

Dengue is a viral infection, which if not managed timely, has grave outcomes potentially resulting in fatality. Several tests aid in diagnosing the infection, but these cannot predict about disease progression towards severe dengue. The current study aimed to investigate the role various hematological and biochemical alterations during the earlier phase of infection in predicting the severity of disease.

This study revealed a predominance of females, constituting 57% of the study participants, whereas males accounted for 43 %contrary to another study[11] however majority of the published studies didn't show any significant differences in gender distribution[16]. In the present study younger age group showed higher incidence of infection. These results align with the outcomes of a previous study[11]. This might be the result of occupational and recreational exposure

A notable abnormality in the hematological profile was thrombocytopenia. A study reported occurrence of thrombocytopenia from the occurrence of symptoms and throughout the progression of disease in severe forms of infection[18]. There could be multiple mechanisms underlying this including damage inflicted to megakaryocytic precursors causing reduced platelets production, immune mediated peripheral destruction due to formation of immune complexes between viral antigens and preexisting antibodies, platelet aggregation and complement mediated lysis[11]. Another significant finding was a higher hematocrit in the infected group, most probably attributable to plasma leakage.

During infection, an acute phase response is triggered. This is accompanied by production of acute phase proteins, out of which "positive phase reactants increase in concentration whereas negative phase reactants decline. Serum ferritin and C Reactive protein (CRP) are positive phase reactants produced by hepatocytes in response to inflammation[17]. The current study revealed higher than normal levels of ferritin and hsCRP. This is supported by other published studies. Study conducted by M Nadeem et al. showed significant correlation between hyperferritinemia on the day of admission and development of severe dengue during the hospital stay[15]. Another study indicates the role of serum ferritin as an early diagnostic and prognostic biomarker .Moreover; a large case control study demonstrated an association between increased hsCRP levels during the first 72 hours of infection and adverse clinical outcomes particularly in children[17].

In this study, Patients infected with the virus have low Vitamin D levels compared to the group negative for infection. These results align with those obtained by Mario et al. in Mexico[19] & Fatima et al[20] in Pakistan. The potential correlation between vitamin D deficiency and development of dengue is neither a new nor a surprising phenomenon, given that there is evidence in literature about antiviral activity of vitamin D against a number of viral pathogens including Herpes, HIV & influenza. Furthermore studies have demonstrated connection between Vitamin D levels & disease progression, mortality rates, helper T cell counts and inflammatory responses in HIV infection[21]. The possible association between vitamin D levels and dengue was further supported by a study conducted by Giraldo et al. demonstrating that exposure of macrophages to high doses of vitamin D, resulted in development of increased resistance towards the virus by them development of increased resistance, towards DENV, in macrophages upon exposure to high doses of vitamin D [22].

Hepatic involvement dengue can range from increased levels of liver enzymes without any symptoms of the disease to life threatening hepatic failure. In the present study significantly elevated GGT levels were found, however there was no significant difference in the levels of AST, ALT, ALP, direct billirubin and total bilirubin, contrary to other studies[9, 23]. Dengue fever is also known to modify host's lipid metabolism. In this study, the alterations in lipid metabolism of the infected individuals included high serum triglycerides and low serum Cholesterol, LDL-C and HDL-C. These results were similar to those obtained by other researches[9, 2]. A viral organelle, composed of different type of lipids is synthesized in host's endoplasmic reticulum, called the replication complex and viral proteins facilitate the uptake of cholesterol by this complex, utilizing FASN and HMGCR. Consequently triglyceride levels are increased whereas cholesterol levels decrease. Hypolipidemia is considered an indicator for the severity of disease.[9]

CONCLUSION

The results from the present study showed significant alterations in the biochemical and hematological profiles of patients infected with DENV compared to negative control group. These findings indicated the potential role of these parameters as prognostic markers to asses the development of severe dengue during early stages of the disease.

Author's Contribution:

Concept & Design of Study:	Erum Amir
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Conflict of Interest: The study has no conflict of interest to declare by any author.

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