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ORIGINAL ARTICLE

Dengue fever and prognostic utility of inflammatory markers.

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ABSTRACT… Objective: To determine presence of inflammatory markers i.e. hyper-ferritinemia, elevated lactate dehydrogenase, and liver enzymes in dengue fever and their correlation with different classes of dengue severity, defined by new WHO classification. **Study Design:** Cross-sectional Analytical study. **Setting:** Medical Department of Dr. Ruth KM Pfau Civil Hospital Karachi. **Period:** October 2022 to March 2023. **Methods:** Conducted in confirmed cases of Dengue fever, after consent all patients were categorized in three i.e. D-W, D+W and SDF groups. **Results:** 120 patients were included in study. 24 suffered from Severe Dengue Fever (SDF) whereas 28 were in Dengue without warning signs (D-W) group and 68 were in Dengue with warning signs (D+W) group. We found a significant association between liver enzymes (SGPT and SGOT) and dengue severity. SGOT was significantly increased in cases with SDF as compared to D-W and D+W cases (p-value = 0.002). Similarly, ferritin was significantly increased in SDF cases compared to non-severe cohort (p-value <0.001). Using the area under the curve (AUC) of ROC curve, ferritin level (cutoff value of 800), produced a sensitivity of 75% for severe dengue fever. Mean length of stay in patients with non-severe dengue group was 3.63 days vs. 4.3 days in those with SDF (P value 0.002). **Conclusion:** As dengue is becoming a huge burden especially in developing countries, need to take proper measures to establish disease severity and according to severity resource allocation is cornerstone. Significant higher ferritin levels in severe dengue fever than other dengue groups, observed in our study, making serum ferritin a surrogate marker for dengue to predict the severity of dengue fever.

Key words: Dengue Without Warning Signs, Dengue with Warning Signs, Ferritin, Lactate Dehydrogenase, Liver Enzymes (SGPT, SGOT), Severe Dengue Fever.

INTRODUCTION

Dengue virus (DENV) being transmitted in humans by bite of female mosquito Aedes Agepti, is a member of the Flaviviridae family; positive-stranded RNA virus. It has four serotypes, DENV 1-4, that are each antigenically different.[1] Approximately 390 million people worldwide are being infected via this virus every year and billions are at risk of contracting the disease.[2] It is an acute febrile illness that usually presents with fever, muscle pain, body ache, and vomiting. This infection can range from mild dengue fever (DF) to dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). Dengue infection's clinical course can be divided into three phases are febrile phase, critical, and recovery phase. In 2009, WHO proposed new categorization for dengue fever i.e. dengue without warning sign, dengue with warning sign and severe dengue.[3] A history of a prior infection with one of the four serotypes significantly raises the risk of developing severe dengue. However, the pathogenesis of severe dengue is attributed to a non-neutralizing antibody-dependent enhancement (ADE) in secondary dengue infection and cytokine dysregulation.[4]

Pakistan has a population of more than 216·5 million people, making it the fifth most populous country in the world. Dengue cases first appeared in Pakistan in 1994, but didn't receive much attention until the mid-2000s, when they started to increase dramatically and led to it's outbreak in the coastal metropolis of Karachi.[5] Pakistan's tropical and subtropical regions are where the dengue virus is found because of favorable

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environmental factors, such as rainfall and average maximum temperature.[6] In Pakistan, the monsoon season brings heavy rains and a rise in dengue cases.[7] Floods make this situation worse as it strike the country almost every year due to heavy rain and land sliding.

It is well known that genetics and nutritional status of host and viral-related variables all interact and contribute in severe clinical dengue fever.[8] According to the WHO recommendations regarding dengue, it's important to keep strict vigilance for warning signs such as prolonged vomiting, abdominal pain, lethargy or restlessness, irritability, giddiness, and oliguria.[3] It's incredibly difficult to predict severity of dengue outcomes because these clinical warning symptoms don't show up until late in the illness.[3,9] The WHO recommended that patient with any warning sign should be hospitalized and managed under strict observation. Therefore, identification of risk factors and biomarkers to predict severity and prognosis is crucial as timely measures could be taken for immediate care and intervention can be taken to hault potentially fatal complications.

Like any other disease, dengue has biomarkers that could be used to recognize infection and serve as road mapp for underlying on going pathological processes, or gauge the severity of infection. These biomarkers include: hematocrit (hct), platelet count, white blood cell (WBC), serum albumin, and hepatic enzymes. However, there is currently a dearth of information available regarding authentic biomarkers thaose can guide clinicians to predict severity and course of dengue fever. Since acute-phase reactant have been the subject of extensive research in humans, particularly as indicators of disease[10], it is vital to confirm the presence of certain acute-phase proteins in samples from cohorts where dengue is an endemic disease. In this study, our main purpose is to propose the potential biomarkers that can predict the prognostic utility in dengue fever, we are assessing biomarkers such as serum ferritin, LDH, and liver enzymes in patients with severe dengue and compare them with non-severe dengue. So that any potential biomarkers could be established to predict severity of dengue in any individual patient and we can take measures prior to storm of complications.

OBJECTIVES

1. To document levels of Ferritin, LDH and Liver enzymes (SGPT and SGOT) in WHO proposed categories of Dengue fever.
2. To document association of these biomarkers with severity of dengue infection.
3. To document prognostic utility of these biomarkers in dengue fever.

METHODS

A cross-sectional, Prospective analytical study, conducted in medical department of Dr. Ruth KM Pfau Civil Hospital Karachi, from October 2022 to March 2023 after approval by the Institutional Review Board (IRB) Committee of Dow University of Health Sciences (IRB-2679/DUHS/Approval/2022/1061).

Consecutive, non-probability sampling technique was used, sample size was calculated using WHO sample size calculator, keeping 13% population prevalence of severe dengue in southeast Asia[11], 95% confidence interval and 6% precision, minimum sample size came out was 120.

DATA COLLECTION

Individuals 18 years and above, with recent fever of ≤ seven days and positive dengue profile either Dengue NS1 antigen or Dengue IgM antibody, were included in the study after informed written consent. Dengue patients with other co-morbidities like CKD, CLD, auto-immune illness and other immune-compromised illness, patient taking steroids or immune-modulators or with history of recent blood transfusion were excluded from the study.

All participants were categorized, into three categories, based on the modified WHO classification 2009, "dengue without warning signs (D-W), dengue with warning signs (D+W) and severe dengue fever (SDF)"[3], depending on participant's clinical presentation. 10 ml Blood sample drawn for CBC, CRP, Serum ferritin, LDH and liver enzyme (SGPT, SGOT) by a professional phlebotomist.

Operational Definition

"New World Health Organization (WHO) classifications of dengue (2009).[3]

1. **Dengue without warning signs: (D-W)**
   Fever and two of the following:
   Nausea, vomiting, Rash, Aches and pains, Leukopenia, Positive tourniquet test
   Laboratory confirmed dengue. With ability:
   • To tolerate adequate volumes of oral fluid replacement
   • To pass urine at least once every 6 hours
2. **Dengue with warning signs (D+W)**
   Patients with any of the following features:
   -At least one of the following warning signs: Abdominal pain or tenderness, Persistent vomiting, Clinical fluid accumulation, Mucosal bleed (gingival bleeding, epistaxis, conjunctival bleeding, hematemesis, melena, freshblood per rectum, hematuria, or vaginal bleeding), Lethargy/restlessness, Liver enlargement >2 cm, Increased Htc with concurrent decrease in platelet count ($\leq$100,000 platelets/mm^3).
   OR
   -At least one comorbid condition such as:
   • Pregnancy or Infancy or Old age or Diabetes mellitus or Renal failure
   OR
   -Social circumstances such as
   • Living alone or Living far from hospital
3. **Severe Dengue fever (SDF)**
   Patients with any of the following features:
   -Severe plasma leakage leading to:
   • Shock or Fluid accumulation leading to respiratory distress
   -Severe bleeding as evaluated by clinician
   -Severe organ involvement:
   • Liver: AsT or AlT > = 1000 IU/L
   • CNS: impaired consciousness."

DATA ANALYSIS

The quantitative variables expressed as mean and standard deviation whereas qualitative variables expressed as frequencies and percentages. Chi-square test was used to calculate the correlation between variables. One-way analysis of variance (ANOVA) used as the statistical test of significance to compare each parameter with different severity groups. The receiver operating characteristic (ROC) curve, used to determine, ferritin and LDH cutoff values for severe and non-severe dengue groups, as well as the test's specificity and sensitivity. AUC values between 0.5 and 0.60, 0.6 to 70, 0.7 to 80, 0.8 to 90, and 0.90 to 1.00 were categorized as failing, bad, fair, good, and excellent, respectively. The level of statistical significance set as p- value < 0.05. 2-tailed hypothesis testing used. SPSS software version 23.0 was used to perform the analysis.

RESULTS

120 patients were included in study. 24 suffered from Severe Dengue Fever (SDF) whereas 28 were in Dengue without warning signs (D-W) group and 68 were in Dengue with warning signs (D+W) group. We found a significant association between liver enzymes (SGPT and SGOT) and dengue severity. SGOT was significantly increased in cases with SDF as compared to D-W and D+W cases (p-value = 0.002). Similarly, ferritin was significantly increased in SDF cases compared to non-severe cohort (p-value <0.001). Using the area under the curve (AUC) of ROC curve, ferritin level (cutoff value of 800), produced a sensitivity of 75% for severe dengue fever. Mean length of stay in patients with non-severe dengue group was 3.63 days vs. 4.3 days in those with SDF (P value 0.002).

DISCUSSION

The symptoms of dengue infection can vary from a self-limiting form to severe multi-organ failure that can cause catastrophic life-threatening conditions such as plasma leakage, hemorrhage, or other serious complications. The prevalence of dengue fever among adults in South-East Asia has significantly increased recently, leading to an increase in hospitalizations and associated costs.[12] Researchers are looking into several clinical profiles and the development of a number of biochemical markers in this sickness, so that promptly disease can be controlled and treated to mitigate risk of complications.

The prevalence of dengue fever in men was 80/120, 66.6% in this study, while a larger percentage of men had D+W symptoms (60/68, 88%).

Disease Severity	Dengue Without Warning Sign (D-W) (n=28)		Dengue With Warning Sign (D+W) (n=68)		Severe Dengue Fever (SDF) (n=24)		Pearson Chi-Square
Statistic	**N**	**%**	**N**	**%**	**N**	**%**	**P-Value**
Age (years)median ±SD	20±9.2		23±10.5		28.5±10.5		<0.001
Gender							<0.001
Male	8	28.5	60	88	12	50	
Addiction	0		12	18	8	33	0.001
Comorbids	0		16	23.5	8	33	<0.001
Clinical features							
Body ache	24	86	60	88	24	100	0.005
Bleeding							<0.001
Minor Bleeding	0	0	52	76	12	50	
Major bleeding	0	0	0	0	12	50	
Abdominal pain	20	71	60	88	24	100	0.003
Loose motions	8	28.5	20	29	4	17	0.463
Vomiting	12	43	52	76	16	67	0.008
Petechiae	12	43	64	100	24	100	<0.001
Ascites	4	14	16	23.5	12	50	0.01
Pleural effusion	4	14	12	18	12	50	0.005
Hospital stay of more than 3days	8	28.5	64	100	24	100	<0.001

Table-I. Baseline demographic details and characteristics of Dengue patients with respect to disease severity

Disease Severity	Dengue Without Warning Sign (D-W)		Dengue With Warning Signs (D+W)		Severe Dengue Fever (SDF)		
Statistic	**Mean± SD**	**Median (IQ Range)**	**Mean± SD**	**Median (IQ Range)**	**Mean± SD**	**Median (IQ Range)**	**P-Value**
Hematological markers							
Hemoglobin (g/dl)	11.2(2.0)	12(3.7)	14.2(1.99)	14(2)	12.1(1.09)	12(2)	<0.001
Absolute Neutrophil count	3505(2134)	2100(4336)	2549(1559)	2400(700)	2754(1444)	2512(1900)	0.114
Absolute Lymphocyets count	1880(760)	1800(1400)	1885(1067)	1680(1240)	2816(2235)	1730(1760)	0.150
Platelet count Day1	126(90)	119(186)	64.4(92)	35(42)	24(18)	18(17)	<0.001
Platelet count Day3	110(64)	94(146)	79(59)	57(52)	60(37)	46(69)	0.004
CRP(mg/dl)	9.6(9.8)	4(19.7)	7.5(9.4)	4(3.1)	14.8(18.4)	7.2(13.6)	0.155
SGPT(IU/l)	53(44)	41(42)	98(63)	74(58)	367(374)	252(270)	<0.001
SGOT(IU/l)	117(118)	84(85)	148(105)	94()	505(285)	428()	<0.001
Ferritin(ng/ml)	1203(2704)	150(71)	1854(1191)	1400(1682)	8040(9269)	4850(1429)	0.006
LDH(IU/l)	367(208)	357(160)	388(108)	380(166)	761(448)	640(315)	0.001

Table-II. Hematological and Biochemical parameters of Dengue patients with respect to Disease Severity

Analysis of mean (robust Welch's test) and variance between non-severe and severe Dengue patients. (p-value less than 0.05)

	AUC [IC=95%]	Cut-off value	Sensitivity [IC=95%]	Specificity [IC=95%]	PPV [IC=95%]	NPV [IC=95%]
CRP	0.62	5.9	66%	71%	25.38%	93.32%
LDH	0.87	427	83%	71%	29.96%	96.55%
Ferritin	0.85	2170	83%	73%	31.48%	96.64%
SGPT	0.80	95	83%	71%	29.96%	96.55%
SGOT	0.91	143	83%	71%	29.96%	96.55%

Table-III. Diagnostic performance of continuous variables and cut-off value

Deshkar et al. likewise found this to be the case in his study.[13] According to a Pakistani research, men make up roughly 68.6% of the population.[14] One explanation for why young men are more affected could be that they are more susceptible to infection due to their outside work. Another claim makes the case that female immune responses are stronger than those of males, which leads to more cytokines being produced and, ultimately, greater resistance to dengue infection. The average age of dengue fever patients in our study was 27 years (SD=10), roughly, and there was a similar pattern of age group being influenced in many other Indian studies as well.[13]

Similar to what Khan et al. had noticed, the three most frequent symptoms in our study were bodyache (90%) abdominal discomfort (86.7%) and vomiting (66.7%).[15,16] In our study, 83.3% of dengue patients had petechial rash, which is comparable to the finding made by Itoda et al.[17] Pleural effusion and ascites were observed in (26.7%) and (23.3%), respectively. Major bleeding was only reported in ten (10) percent of patients, but minor bleeding was seen in 53.3 percent of patients. In cases of severe dengue fever, petechial rash, fluid third spacing, and bleeding were more frequent.

The reticuloendothelial system of our bodies is harmed by dengue virus. Ferritin and lactate dehydrogenase (LDH) are indicators of multi-organ damage and reticuloendothelial system activation. Although dengue is a non-hepatotrophic virus, hepatic enzyme dysregulation is frequently seen, since the liver is a crucial component of the reticuloendothelial system. In general, AST levels are more affected than ALT levels.[18,19,20]

According to table2, severe dengue patients had median serum ferritin levels 4850 g/dl (IQR=14290), that was significantly high than non-severe cases. Our results are in line with those of other studies[21], that found ferritin to be a reliable biomarker for predicting severe dengue. With an area under the curve (AUC) of 0.863, a standard error (SE) of 0.043, a 95% confidence interval (CI) of 0.778 to 0.947, and a cut-off level of 2170, serum ferritin level was a “good” predictor of severe dengue in our study. This predictor had an 83% sensitivity and 73% specificity for diagnosing severe dengue.

LDH, another biomarker assessed in our study for predicting severe dengue with an AUC of 0.87 turned out to be a significant biomarker. Although there was no statistical significant difference found in ldh levels between non-severe without warning sign (D-W) and non-severe with warning (D+W) sign groups (p-value0.879), but a statistical significant difference identified between non-severe with warning(D+W) signs and severe dengue fever (SDF) (p-value0.001).

In contrast to previous studies, our study found CRP, not a reliable indicator of dengue severity.[22] The CRP level did not significantly differ between the D-W, D+W, and SDF groups (p-value 0.155), making it an ineffective marker in this study for determining severity of dengue infection.
With an area under the curve (AUC) of 0.91 and a cut-off level of 143, SGOT was a “very-good” predictor of severe dengue in this research, therefore making hepatic impairment a well-known aspect of dengue infection. SGOT had an 83% sensitivity and 73% specificity for identifying severe dengue. Similar results were found in other old researches.[23] Raised aminotransferases were also noted by Souza et al.[24] who discovered that AST levels were higher than ALT levels in their study.

Limitations: The participants included were from single-center and all were in-patient or hospitalized. Inflammatory markers were only evaluated on the first day of hospitalization; correlations with severity were not determined by serial measurements. A large sample size, several locations, and repeated testing of all inflammatory markers should be done in order to generalize, further implement and clearly demonstrate the results.

CONCLUSION

In people suffering and hospitalized due to dengue infection, our study revealed number of clinical variables that were each individually attributed to the emergence of severe dengue. In

order to lower morbidity and mortality, this can help in the early identification and timely therapy of individuals who are at risk. In our study, we explored that severe dengue fever patients had significantly higher serum ferritin levels than other dengue groups, making serum ferritin a surrogate marker for dengue to predict the severity of dengue fever infection.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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3	Darshan Kumar	Design, Supervision & critical review.	
4	Zill-e-Huma	Data collection and literature review.	
5	Gul Anum	Data collection, Analysis and literature review.	
6	Rashid Qadeer	Conception, design and supervision, Critical review.	