The clinical profile, hematological parameters and liver transaminases of Dengue NS1 Ag positive patients admitted to Jaffna Teaching Hospital, Sri Lanka

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Research note

Keywords: Dengue, NS1 antigen, Hematological parameters, Transaminases

Posted Date: July 2nd, 2019

DOI: https://doi.org/10.21203/rs.2.10807/v1

Version of Record: A version of this preprint was published on September 23rd, 2019. See the published version at https://doi.org/10.1186/s13104-019-4655-8.

Abstract

Objective Objective of the study is to evaluate the symptoms and signs and nonspecific clinical and laboratory parameters of the dengue NS1 positive patients on the admission from day1 to day 5 at medical wards of Jaffna Teaching Hospital. Results Blood samples were collected from 150 consecutive suspected dengue patients from day 1 to 5 of the illness. Seventy-eight patients were positive for Dengue NS1. Patients who had NS1 positivity after 3days of illness suffered from severe form of disease and patients with severe thrombocytopenia had significant rise in hemoglobin, hematocrit and liver transaminase levels which are considered as severe form of disease. Severe thrombocytopenia patients had complicated illness. This shows longevity presence of NS1 antigen could lead to severe form of dengue and could be a prognostic marker of dengue outcome.

Introduction

Dengue Fever (DF) is an arboviral disease which is caused by one of the four antigenically distinct virus serotypes[1]. In recent years, dengue has become a major global public health concern. According to Epidemiology Unit Sri Lanka year 2017 has been recorded as the highest number of dengue suspected cases ever with the number of 186101 suspected cases[2].

Dengue manifests wide range of clinical spectrum from asymptomatic fever to life threatening dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), as there is no licensed vaccine, fluid management and monitoring for complications, is the only available option[3]. Early diagnosis and management of cases plays a crucial role in preventing the severity as well as fatality of dengue cases. Physicians in state health sector follow the dengue management guidelines developed by the Ministry of Health, Sri Lanka, in collaboration with WHO [4]. The wide range of signs and symptoms associated with severe dengue virus (DENV) infection and the identification of those that are highly likely to a major clinical challenge. Therefore, confirmation of dengue is achieved by specific tests, nonspecific tests and nonspecific clinical parameters [5].

The Dengue virus non-structural protein 1(NS1) antigen test is the specific dengue test, available for the use of early diagnosis of dengue with significant sensitivity and specificity [6]. Dengue NS1 antigen is a glycoprotein, produced both in membrane associated and in secretory forms, is detectable in blood at high concentration in sera of dengue infected patients during the acute clinical phase of dengue. It could be detected from day 1 up to day 5 with high sensitivity and it may be extended up to 9 days after the onset of infection in some cases. It is also detectable in both primary and secondary dengue infections[3]. As other dengue confirmatory tests require a well-equipped laboratory, rapid NS1 test can be performed in a hospital with lack of laboratory facility. Apart from the dengue specific parameters, platelet count is a prognostic laboratory parameter which is available to identify severity of dengue, even though thrombocytopenia is not an early indicator of severe dengue, but it helps in predicting the progression of disease [7, 8].

Objective

In this study we evaluated the symptoms and signs and nonspecific clinical and laboratory parameters of the dengue NS1 positive patients on the admission from day1 to day 5 at medical wards of Teaching Hospital Jaffna, the only tertiary care center of the region.

Methodology

Ethical clearance was obtained from, Ethical review committee, Faculty of medicine, University of Jaffna. This study was carried out in Jaffna Teaching Hospital from October 2017 to May 2018.With informed written consent, blood samples

were collected from 150 fever patients from day 1 to 5 who appeared to have Dengue fever under Dengue guideline of the Ministry of health, Sri Lanka and rapid NS1 antigen test (RapiGEN BIOCREDIT, Republic of Korea) performed.

Detailed clinical examination and history taking was performed using questionnaire and non-specific laboratory tests including total white blood cell (WBC) count, platelet count, hemoglobin (Hb), hematocrit (HCT), and liver function tests were obtained from bedhead tickets of the all the participating patients on the day of admission. Patients with severe thrombocytopenia were assessed for leakage; ultrasound scanning was performed to see any sign of fluid accumulation either in the pleural space or in the abdominal cavity.

Nonspecific laboratory parameters of the dengue NS1 positive patients were analyzed in groups according to the day of NS1 was positive on admission. For each group which is separated according to the day mean and standard deviation were calculated. All NS1 positive patients were further divided in to two groups (group A and group B) according to their platelet count on the day of admission. Group A is defined as the group of patients whose platelet count above 100 $x10^9$/L (N = 37) and group B is defined as the group of patients with platelet count below 100 $x10^9$/L (N= 41). t- Test was performed and p value less than 0.05 were considered as significant.

Results

One hundred and fifty consecutive patients who were admitted to the medical ward of teaching hospital with clinically suspected dengue fever tested for NS1Ag as they had duration of illness ≤ 5 days. Out of 150 patients, 78 patients were positive (male -54 and female - 24) for dengue NS1 Ag. The highest numbers of dengue NS1 positive patients were from age group 21- 30. Fever was the most common symptom found in all the patients and other symptoms are headache (80.7%), nausea (56.4%), anorexia (53.8%), vomiting (52.5%), myalgia (50%), abdominal pain (29.4 %) and retro orbital pain (17.9%).

Table 1: Distribution of mean non-specific laboratory parameters of NS1 positive patients (Patients who admitted from Day 1 – Day 5)

For the dengue NS1 positive patients, leucopenia (< 5 x 10^9/L) was observed in 67 out of 78 patients (85.8%) from day 1 -5. The lowest mean WBC value was observed on people who got admitted on the day 5 onset of illness 2.66 x 10^9/L (Table 1). The mean WBC count 3.76x 10^9/L and 3.26x 10^9/L were observed between group A and B. Neutropenia (<2 x 10^9/L) was observed on the patients after 3 days onset of illness. 33 patients had neutropenia from day 3 to day 5 except one from day 1. Lymphocytopenia (<1x 10^9/L) was widely observed, there were 58 (74.3%) patients who had lymphocytopenia from day 1 to day 5 onset of illness. The lowest mean value of lymphocyte 0.77 x 10 ^{9}/L was observed on day 2. There was no statistical significance observed between group A and B in the WBC, neutrophil and lymphocyte counts (Table 3).

In the laboratory parameter, mean hemoglobin value for all 5 days were within the reference range, 13.8 mg/dL was recorded as the maximum mean value of hemoglobin level. Only 4 patients had the hemoglobin above 16 mg/dL (Table 2) but the hemoglobin value of group A and group B patients are statistically significant <0.05 (Table 3). The maximum mean hematocrit percentage was observed as 40.7% on day 4, mean HCT value for group A and B are 37.7 and 40.6 respectively which is statistically significant. There were 17 patients who had raised hematocrit concentration by 10% and only 3 patients who had increased hematocrit percentage by 20% from the normal range (Table 2).

Table 2: Distribution of laboratory dengue feature of dengue NS1 positive patients

Thrombocytopenia (< 150x 10^9/L) was the most common abnormal laboratory parameter which was observed in 66 patients (84.6%) and 41of them (52.5%) suffered from severe thrombocytopenia (<100 x 10^9/L). The minimum mean

value of the platelets was recorded on day 4 as 76.64 x 10^9/L, and all 13 patients from day 5 onset of illness suffered from thrombocytopenia. The mean value of platelet count of group A and B were 144.43x 10^9/L and 60.04x 10^9/L which has strong statistical significance (<0.001).

Table 3: Distribution of laboratory parameters between group A and B (t – test)

When elevated mean serum transaminase levels (ALT >63 U/L and AST > 37 U/L) taken into consideration, 32 patients had elevated ALT level above reference range and 63 patients had elevated AST level above the reference range. The mean maximum value of ALT and AST was observed on day 5 as 94 U/L and 116 U/L and the mean ALT and AST value for group And B were 69.1 and 98.8, 64.1 and 122.6 respectively (Table 3). Among 78 NS1 positive patients, 09 had the evidence of leakage and all of them had severe thrombocytopenia and elevated transaminase levels. The mean platelet count, ALT and AST level were 58.9 x 10^9/L, 108.3 U/L and 149.6 U/L respectively.

Discussion

In our study day 1 is considered as the day which the patient had felt fever and followed with other clinical symptoms. The age groups between 12 – 20 and 21 – 30 years combined were 64.1% of the total dengue NS1 positive cases (see Additional File 1: Table S1; https://figshare.com/articles/Additional_File_1_doc/8325680). More than 50% of NS1 dengue positive patients in our study had symptoms such as headache, myalgia, nausea, vomiting and anorexia. Comparatively few patients had diarrhea, retro orbital pain and back pain (see Additional File 2: Table S2; https://figshare.com/articles/Additional_File_2_doc/8325731).

Leukopenia is a well-established feature of dengue which is due to the direct marrow suppression by the dengue virus [5, 9]. In our study the mean WBC count was gradually decreasing with the day of onset of illness. The mean ratio of neutrophil to lymphocytes were >1 for day 1- 5 on admission (Table 1). In year 2018 similar observation reported in the Thailand dengue patients, it also stated from day 6-9 the ratio was reversed [10]. Even though dynamic changes of the mean value for WBC, neutrophil and lymphocytes observed in the dengue NS1 positive patients observed according to the day of NS1 positive, there were no statistical significance observed in WBC, neutrophil and lymphocytes between group A and group B.

Dengue NS1 positive patients in this study could not be further classified apart from dengue fever due to the lack of raised hematocrit level and absence of leakage even in severe thrombocytopenia. Considering the complexity of WHO classification of dengue severity, we adhered to the platelet count as a single marker of dengue severity [11-13]. Thrombocytopenia is a common clinical condition found in dengue and a predictive biomarker for the severity of dengue[7, 8, 14]. More than 80% of the total patients had thrombocytopenia in this study and prominent significance were found between group A and B. Platelets plays a role in increased vascular permeability due to the inflammation dependent release of IL-1β [15]. In our study, 9 patients who had severe thrombocytopenia had the sonographic evidence of leakage. There are studies suggesting that anti NS1 antibodies also could play a role in plasma leakage [16, 17].

The mean hemoglobin and hematocrit values lie within the normal reference range. When we consider individually, there were 4 patients from day (3-5) had elevated hemoglobin level above the reference range, 17 patients had risen hematocrit by 10% and 3 patients had risen in hematocrit by 20%.

In group A and B the Hb level and HCT were statistically significant ($P < 0.01$). The patients who had raised HCT over 20% had sonographic evidence of leakage as well. Sudden drop in platelet count and rising hematocrit, are markers for the progression of plasma leakage [5]. In our study, leakers have considerable drop of the mean platelets value when compared to non-leakers, but not all the severe thrombocytopenia patients had developed the leakage.

In our study, the raised aspartate transaminase (AST) levels were found in 63 (80.7%) patients and 32 (52.5%) patients had elevated alanine transaminase (ALT) level. The mean ALT for group A and B are 69.1 U/L and 98.8 U/L, even though the value of ALT level in group B is arithmetically high no statistical significance was found. The mean AST value for group A and B were 64.1 U/L and 123 U/L which has strong statistical significance (P <0.001). In group A 6 patients out of 37 and in group B 15 out of 41 had AST: ALT ratio greater than 1.5. According to a study by Ho and his group in year 2013 elevated aminotransferase (AST/ALT >1.5) in combination with thrombocytopenia and leukopenia serve as a strong nonspecific predictive marker for dengue [18]. In our study, most of the patients had elevated AST than ALT which could be due to extrahepatic release of AST, because AST has various sources such as, heart, striated muscle, erythrocytes, apart from liver but ALT is primarily hepatic origin[19]. Therefore, elevated amount of AST not always truly reflects the hepatic involvement. Moreover, patients with high levels of enzymes may be labeled as severe disease without any effect on the final outcomes [20].

Conclusion

NS1 is a well-known early diagnostic marker of dengue. The longevity of the presence of NS1 antigen in patients with dengue could predict the adverse outcome of the dengue in particular thrombocytopenia and leakage. The leakers are found to be having significant thrombocytopenia and had elevated liver transaminases without overt clinical evidence of shock.

Limitations

The study was only in Adult population who were above years 12 from a single center. The observations and interpretations could vary in a pediatric population.

Abbreviation

NS – Nonstructural

Ag – Antigen

DF - Dengue Fever

DHF - Dengue hemorrhagic fever

DSS - Dengue shock syndrome

DENV – Dengue virus

WBC - White blood cell

Plt - Platelet

Hb - Hemoglobin

HCT - Hematocrit

AST - Aspartate transaminase

ALT - Alanine transaminase

Declarations

Acknowledgements

Authors are grateful to all participants who participated in this study, consultant physicians, medical officers and nursing officers of Jaffna Teaching Hospital for the support given to the study.

Ethics approval and consent to participate

Ethical approval for this research work has been obtained (J/ERC/17/80/DR/0040) from the Ethical Review Committee (ERC) of the Faculty of Medicine, University of Jaffna, Sri Lanka. Written consent from participants were obtained to participate in this study.

Consent for publication

Not Applicable

Availability of data and material

Individual patient clinical details and parameters are not publicly available, but the data sets analyzed during this study are available from the corresponding author on reasonable request by emailing the corresponding author.

Funding

Financial assistance from the National Science Foundation of Sri Lanka (RPHS/2016/D02)

Authors' contributions

SNS and TK designed the study. TTPJ carried out sample collection and laboratory studies. TTPJ, TK, VA, KG and SNS performed data analysis. TTPJ, TK and SNS drafted the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Additional Files

Additional Files

Additional File 1: Table S1.doc

https://figshare.com/articles/Additional_File_1_doc/8325680

Age distribution of NS1 positive patients

Additional File 2: Table S2.doc

https://figshare.com/articles/Additional_File_2_doc/8325731

Clinical characteristics of the dengue NS1 patients (Day 1 – Day 5)

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Tables

Table 1: Distribution of mean non-specific laboratory parameters of NS1 positive patients (Patients who admitted from Day 1 – Day 5)

Parameters	Day 1	Day 2	Day 3	Day 4	Day 5
WBC $x10^9$/L	5.657 ± 0.93	5.027 ± 1.65	3.562 ± 1.56	3.312 ± 1.672	2.67 ± 1.03
Neutrophil $x10^9$/L	3.380 ± 0.35	3.82 ±1.213	2.39 ± 1.14	1.943 ± 0.98	1.66 ± 0.69
Lymphocyte $x10^9$/L	1.290 ± 0.52	0.77 ± 0.411	0.787 ± 0.53	1.013 ± 0.87	0.69 ± 0.37
Hb mg/dL	12.56 ± 1.22	12.37 ± 1.15	13.08 ± 2.11	13.78 ±1.85	13.07 ± 1.46
HCT%	39.67 ± 3.50	37.29 ± 2.88	38.40 ±5.40	40.71 ± 5.16	38.49 ± 4.31
Plt $x10^9$/L	249.3 ± 28.1	145.7 ± 26.6	101.2 ± 53.5	76.64 ± 43.28	89.23 ± 25.09
ALT U/L	27.0 ± 4.36	46.29 ± 14.50	86.6 ± 64.0	94.0 ± 93.5	94.7 ± 98.3
AST U/L	33.0 ± 5.00	44.0 ± 16.25	80.7 ± 52.8	116.8 ± 92.5	118.8 ± 102.4

Table 2: Distribution of laboratory dengue feature of dengue NS1 positive patients

Parameters		Day 1	Day 2	Day 3	Day 4	Day 5	Total
Leukocytes (<5 $x10^9$/L)		2/3	4/7	24/27	25/28	12/13	67/78 (85.8%)
Neutrophil (< 2 x 10^9/L)		1/3	-	10/27	14/28	09/13	34/78 (43.5%)
Lymphocytes (<1 x 10^9/L)		1/3	5	22/27	19/28	11/13	58/78 (74.3%)
Hb (>16 mg/dL)		-	-	1/27	2/28	1/13	4/78 (5.1%)
HCT (39-38 %)	20% Increase	-	-	1/27	2/28	-	3/78 (3.8%)
	10%Increase	-	-	4/27	11/28	2/13	17/78 (21.7%)
Platelets	(<150 $x10^9$/L)	-	4/7	08/27	08/28	05/13	25/78 (32%)
	(<100 $x10^9$/L)	-	-	15/27	18/28	8/13	41/78 (52.5%)
ALT (>63 U/L)		-	1/7	14/27	12/28	5/13	32/78 (41%)
AST (>37 U/L)		1/3	5/7	21/27	26/28	10/13	63/78 (80.7%)

Table 3: Distribution of laboratory parameters between group A and B (t – test)

FBC	Group A/ Group B	No.of Patients	Mean Std.Dev	*P* value
WBC $x10^9$/L	Group A	37	3.76 ± 1.66	0.21
	Group B	41	3.29 ± 1.60	
Neutrophil $x10^9$/L	Group A	37	2.49 ± 1.32	0.088
	Group B	41	2.04 ± 0.955	
Lymphocyte $x10^9$/L	Group A	37	0.776 ± 0.372	0.23
	Group B	41	0.955 ± 0.830	
Hb (mg/dL)	Group A	37	12.7 ± 1.24	0.0067
	Group B	41	13.8 ± 2.13	
HCT%	Group A	37	37.7 ± 3.54	0.0094
	Group B	41	40.6 ± 5.65	
Plt $x10^9$/L	Group A	37	144 ± 44.3	<0.0001
	Group B	41	60.0 ± 26.3	
ALT (U/L)	Group A	37	69.1 ± 72.8	0.100
	Group B	41	98.8 ± 83.1	
AST (U/L)	Group A	37	64.1 ± 65.2	0.0008
	Group B	41	123 ± 81.7	