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"Pattern of Hematological Abnormalities in NS1 Positive Dengue Fever: A Study in a Tertiary Care Hospital"

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Abstract | **Original Research Article**

Introduction: Dengue infection is an arthropod borne disease caused by Dengue virus in humans. Dengue virus infection has more potential to produce severe form of the disease with more severe symptoms. Proper diagnosis of dengue fever is very important for its safe management. NS1 antigen test (nonstructural protein 1), is a test for dengue, introduced in 2006. It allows rapid detection on the first day of fever, before antibodies appear some 5 or more days later. ***Aim of the study:*** The aim of this study was to determine the pattern of hematological abnormalities in patients with NS1 positive dengue fever. ***Methods:*** It was a cross sectional study which was conducted among 300 patients tested for dengue admitted in the Department of Medicine, Sheikh Sayera Khatun Medical College, Gopalgonj from January 2017 to December 2017 with symptoms related to dengue fever. This study was approved by the ethical committee of the respective hospital previously. Proper written consents were obtained from all the patients with NS1 positive dengue fever before starting the intervention. ***Result:*** In our study it was found that, 90.8 % patients had primary dengue infection and 35.5% patients had secondary dengue infection. Most patients were between the ages of 10-20 years (26%), among them 19.7% were having primary dengue infection. Among 10-20 years of age 50% female patients were with false dengue. Among total NS1 positive patients platelet count was <150 x109/L in 30.43% (n=49) and <100 x109/L in 49.69% (n=80) patients. Out of total 161 participants 22.98% had thrombocytopenia Grade 1 which was the highest portion. Then 14.29% had Grade 2, 7.45% had Grade 3 and 3.73% had Grade 4 thrombocytopenia. Neutropenia (<2 x 109/L) was observed on 23 patients who had neutropenia in febrile phase and 43 had in critical phase. The mean value of neutrophil in febrile phase was 2.54 and in critical phase it was 1.76 which was statistically highly significant. Lymphocytopenia (<1x 109/L) was widely observed in both febrile phase and critical phase and there was no statistical significance observed between febrile phase group and critical phase group. ***Conclusion:*** To prevent dengue fever awareness among people, government as well as the physicians is a must. Although it is said that, knowledge does not necessarily lead to good practice, we think knowledge is very essential for preventing such a diseases which may be cause of an epidemic in anywhere anytime.

Keywords: Hematological Abnormalities, NS1 Positive, Dengue Fever, Antigen.

INTRODUCTION

Dengue fever, which was once confined to Southeast Asia, has now spread to Southern China, countries in the Pacific Ocean and America, and might pose a threat to Europe. In the last 50 years, dengue virus infections had expanded to many other countries with significant increasing cases[1] up to 2.5 billion people living in endemic countries where about 1.8 billion (more than 70%) in Southeast Asia and the Western Pacific Region[2]. About 50 million dengue infections occur in every year [2], and approximately 500,000 patients are hospitalized of whom dominated by children [2]. Dengue manifests an array 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, are 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. In order

to facilitate the clinical management WHO has proposed 3 dengue clinical phases based on the days of onset of dengue illness, febrile phase / viremic phase (days 1 -3), critical phase (days 4-6) and recovery phase (days >7). However, it is not true always; sometimes prolonged febrile phase is followed by the recovery phase in some dengue patients, while the febrile and critical phases may overlap in some others patients. Plasma leakage is the pathological hallmark of the beginning of critical phase if there is no plasma leakage it is still considered as prolonged febrile illness [4]. Physicians in the state health sector follow the dengue management guidelines developed by the Ministry of Health, Sri Lanka, in collaboration with WHO [5]. The wide range of signs and symptoms associated with severe dengue virus (DENV) infection and the identification of those that are highly likely to be a major clinical challenge. Therefore, confirmation of dengue is achieved by specific tests, nonspecific tests and nonspecific clinical parameters [6]. The dengue virus non-structural protein 1(NS1) antigen test is the specific dengue test, widely applied for early diagnosis of dengue with significant sensitivity and specificity [7]. 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 early febrile phase. 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]. Although 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 [8]. In preventing dengue fever awareness of people about the disease is a must. But till now the awareness level is not up to the mark. Some studies found about one-third adult population with sufficient knowledge about dengue fever and its fast spreading to other people. However, only about 17% of them aware and clearly know the way for prevention [9].

OBJECTIVES

a) General objective

- To determine the pattern of hematological abnormalities in patients with NS1 positive dengue fever.

b) Specific Objectives

- To evaluate the laboratory findings of dengue NS1 positive patients.

METHODOLOGY & MATERIALS

It was a cross sectional study which was conducted among 300 patients tested for dengue admitted in the Department of Medicine, Sheikh Sayera Khatun Medical College, Gopalgonj from January 2017 to December 2017 with symptoms related to dengue fever. This study was approved by the ethical committee of the respective hospital previously. Proper written consents were obtained from all the patients with NS1 positive dengue fever before starting the intervention. The dengue onset day (day 1) referred to the day in which symptoms suggestive of dengue (i.e., fever, retro-orbital pain, headache, arthralgia and/or myalgia) started developing; this day was identified based on the patient's description at history taking upon his or her hospital presentation. All the NS1 positive patients with febrile illness were included in the study. Detailed clinical examination and history taking was performed using a structure-questionnaire and results of non-specific laboratory tests including total white blood cell (WBC) count, platelet count, hemoglobin (Hb), hematocrit (HCT), and liver enzymes tests were obtained from bedhead tickets of all NS1 positive participating patients on the day of admission. Patients with severe thrombocytopenia (below 100 x109/L) 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. DEN NS1 positive patients were separated into 2 groups according to the clinical phases (febrile 1-3 days, critical 4-5 days) based on the days onset of illness during the admission day. Leaking patients were extracted and analyzed with DEN NS1 on day 4-5 without leakage. On the basis of platelet count, the cases were divided into the four following grades: Grade 1: 75-150 × 10^3/µL, Grade 2: 50-75 × 10^3/µL, Grade 3: 25-50 × 10^3/µL, Grade 4: <25 × 10^3/µL where µL indicates micro liter. For separated groups nonspecific laboratory parameters were compared using the Student's-t-test and a 2-tailed $p < 0.05$ was considered statistically significant. In total 300 consecutive patients who were admitted to the Department of Medicine of mentioned hospital with clinically suspected dengue fever were tested for NS1Ag as they had duration of illness ≤ 5 days. In collecting data MS-Excel in analyzing data SPSS version 20 and in displaying data several tables were used.

RESULT

In this study the total study people ware 300 in number. Among them 161 participants were found as NS1 positive which was 53.67% out of the total population was. Among those 161 NS1 positive cases 77 (47.83%) were in febrile phase where 63.46% were male and 36.36% were female. On the other hand, 84 out of 161 NS1 positive patients were in critical phase which was 52.17% containing 64.28% male as well as 35.71% female. So among all the NS1 positive patients

103 (63.97%) were male and 58 (36.03%) were female. In analyzing the sign and symptoms of the NS1 positive patients we found the highest number of patients suffered from fever which was 83,85% in total. Then 67.08% suffered from headache, 48.45% from nausea, 45.96% from anorexia, 40.37% from vomiting, 38.51% from myalgia, 26.09% from abdominal pain, 16.77% from retro orbital pain, 12.42% from arthralgia and 9.32 from back pain. In analyzing laboratory findings among Febrile phase and critical phase group patients we found significant correlation in mean leukocytes x109/L and mean AST (U/L) where the p value was <0.05. On the other hand among leakage and non-leakage groups we found significant correlation in mean Hb (mg/dL) and mean HCT% where the p value was < 0.05 also. Evidence of leakage was found in 19 patients which was 11.80% among the total 161 NS1 positive cases. Among those 19 patients 7 from day-3 and 12 patients from day-4 onset of illness found with leakage. Most of the leaking patients had symptoms like fever, head ache, persistent vomiting, abdominal pain and anorexia. During the febrile phase of the dengue NS1 positive patients, leucopenia (< 5 x 109/L) was observed in 62 out of 77 patients (80.52%) and in critical phase 37 out of 84 patients it was in 69 (82.14%). The mean WBC count 3.86 x 109/L and 3.21 x 109/L were observed between febrile phase and critical phase which is statistically significant. Neutropenia (<2 x 109/L) was observed on 23 patients who had neutropenia in febrile phase and 43 had in critical phase. The mean value of neutrophil in febrile phase was 2.54 and in critical phase it was 1.76 which was statistically highly significant. Lymphocytopenia (<1x 109/L) was widely observed in both febrile phase and critical phase and there was no statistical significance observed between febrile phase group and critical phase group. Among total NS1 positive patients platelet count was <150 x109/L in 30.43% (n=49) and <100 x109/L in 49.69% (n=80) patients. Among 80 patients with platelet count <100 x109/L, 78 were with several grades of thrombocytopenia. In our study out of total 161 participants 22.98% had thrombocytopenia Grade 1 which was the highest portion. Then 14.29% had Grade 2, 7.45% had Grade 3 and 3.73% had Grade 4 thrombocytopenia. During day 3-5, NS1 positive patients and leaking patients had no statistical significance in leukocytes count, neutrophil count and lymphocyte count.

Table-I: Distribution of the participants according to NS1 test, stage of diseases and gender (n=300)

Total Participants (300)				
NS1 (-) Ve 139(46.33%)	NS1 (+) Ve 161 (53.67%)			
	Febrile Phase 77 (47.83%)		Critical Phase 84 (52.17%)	
	Male	Female	Male	Female
	49(63.64%)	28(36.36%)	54(64.28%)	30(35.71%)

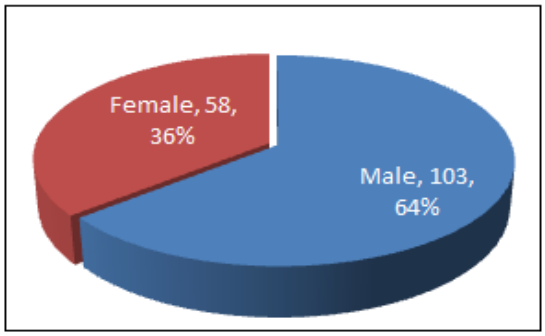

Fig-I: Gender distribution of NS1 positive patients (n=161)

Table-II: Clinical characteristics of the dengue NS1 positive patients from Day 1 to Day 5 (n=161)

Signs/Symptoms	Viremic (N= 77)	Critical (N=84)	Total	%
Fever	62	73	135	83.85
Headache	51	57	108	67.08
Nausea	37	41	78	48.45
Anorexia	35	39	74	45.96
Vomiting	32	33	65	40.37
Myalgia	30	32	62	38.51
Abdominal pain	21	21	42	26.09
Retro orbital pain	13	14	27	16.77
Arthralgia	11	9	20	12.42
Back pain	7	8	15	9.32

Table-III: Laboratory findings of the NS1 positive patients (n=161)

Parameters	Febrile phase Day (1-3) n=77	Critical phase Day (4-5) n=84	P value	Non-leakage n=142 Day (3-5)	Leakage n=19	P value
Mean leukocytes x10^9/L	3.86± 1.71	3.21± 1.62	0.035	2.78± 1.61	4.05 ± 1.03	0.082
Mean neutrophil x10^9/L	2.54 ± 1.32	1.76 ± 1.72	0.075	1.83 ± 0.89	2.53 ± 0.83	0.076
Mean lymphocytes x10^9/L	0.81 ± 0.52	0.87 ± 0.47	0.074	0.87 ± 0.62	1.27 ± 0.73	0.091
Mean Hb (mg/dL)	13.11 ± 1.84	12.87 ± 1.62	0. 092	12.83 ± 1.73	15.03 ± 1.79	0.048
Mean HCT%	37.14 ± 5.04	41.18 ± 5.03	0.082	38.67± 4.21	45.18 ± 4.04	0.035
Mean Plt x10^9/L	120.51 ± 64.29	81.73 ± 37.38	0.064	82.49± 37.11	58.17 ± 27.92	0.193
Mean ALT (U/L)	75.27 ± 57.71	92.34 ± 93.68	0.081	93.33 ± 95.17	106.49 ± 63.12	0.097
Mean AST (U/L)	70.31 ± 48.29	114.17± 95.91	0.041	109.36 ± 98.85	147.36 ± 69.17	0.081

Table-IV: Distribution of laboratory dengue characteristics of dengue NS1 positive patients (n=161)

Parameters		Febrile Day (1-3) n=77		Critical Day (2-5) n=84		Total n=161	
		n	%	n	%	n	%
Leukocytes (<5 x10^9/L)		62	80.52	69	82.14	131	81.37
Neutrophil (< 2 x 10^9/L)		23	29.87	43	51.19	66	40.99
Lymphocytes (<1 x 10^9/L)		58	75.32	56	66.67	114	70.81
Hb (>16 mg/dL)		2	2.60	6	7.14	8	4.97
HCT	20% Increase	2	2.60	4	4.76	6	3.73
(39-38 %)	10%Increase	8	10.39	24	28.57	32	19.88
Platelets	(<150 x10^9/L)	25	32.47	24	28.57	49	30.43
	(<100 x10^9/L)	31	40.26	49	58.33	80	49.69
ALT (>63 U/L)		31	40.26	32	38.10	63	39.13
AST (>37 U/L)		56	72.73	68	80.95	124	77.02

Table-V: Grade distribution of thrombocytopenia among total NS1 positive participants (n=161)

Grade	n	%
Grade 1	37	22.98
Grade 2	23	14.29
Grade 3	12	7.45
Grade 4	6	3.73
Total	**78**	**48.45**

DISCUSSION

In our study fever is the most common symptom found in both febrile and critical phase and more than 50% of NS1 positive febrile phase and critical phase patients had other symptoms such as headache, myalgia, nausea, vomiting and anorexia. Leakage is the hallmark of the beginning of critical phase; only 7 patients had the evidence of leakage during the clinical presentation on the admission day and in other 12 patients it taken more time. In normal case scenario plasma leakage occurs as temperature begins to defervesce, rarely in this study all leaking patients had fever (T> 100.4 F). Leukopenia is a well-established feature of dengue which is due to direct marrow suppression by the dengue virus [10]. In this present study leukopenia was found in 81.37% of the NS1 positive patients. Statistical significance was observed between febrile phase and critical phase in leukocyte count and neutrophil count. Lymphocytopenia was widely observed than neutropenia, the mean ratio of neutrophil to lymphocytes were >1 in both febrile phase and critical phase and there is no significance was observed between febrile phase and critical phase. Similar observation was reported among Thailand dengue patients who initiated this from day 6-9 (recovery phase) and the ratio was reversed [11]. Dengue NS1 positive patients in this study could not be classified as severe dengue apart from dengue fever due to the lack of raised hematocrit level and absence of leakage even in severe thrombocytopenia due to the complexity of WHO classification of dengue severity [12]. Thrombocytopenia is a common clinical condition found in dengue and a predictive biomarker for the severity of dengue [13]. In our study about all the patients had thrombocytopenia in this study and all 19 leaking patients on the admission day had suffered from severe thrombocytopenia. Even though prominent statistical significance found between febrile phase and critical phase NS1 positive patients there was no statistical significance observed between day 4-5 patients' group without leakage. Plasma leakage occurs due to the increased vascular permeability and platelets plays a role in increased vascular permeability due to the inflammation dependent release of IL-1β [14]. Some studies suggest that anti NS1 antibodies could also play a role in plasma leakage [15]. Sudden drop in platelet count and rising hematocrit, are markers for the progression of

plasma leakage [6]. Apart from four NS1 positive patients other patients did not have elevated hemoglobin level above the reference range. Both Hb level and HCT were not statistically significant between febrile phase and critical phase but those parameters were significant between the group of leakers and 4-5 days patients without leakage. As parameters Hb and HCT were taken on the day of admission it may represent the precise Hb and HCT level, because according to the dengue management guideline if the patient's platelet count goes below 100 $x10^9$/L intravenous fluid management has to be started[5]. The infusion of fluid might manipulate the Hb and HCT so inward patients with intravenous fluid management Hb and HCT value might mislead the fact of leakage. In our study, the raised aspartate transaminase (AST) levels were found in 77.02% patients, among these 56 patients were in febrile phase and 68 patients in critical phase. Elevated alanine transaminase (ALT) level was found in 39.13% patients. Among them 31 in febrile phase and 32 in critical phase. Statistical significance was found in transaminases level among the patients representing the two phases. 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, and erythrocytes apart from liver but ALT is primarily hepatic origin [16]. Therefore, elevated amount of AST not always truly reflects the hepatic involvement of the patients. Moreover, patients with high levels of enzymes may be labeled as severe disease without any effect on the final outcomes [17]. Although in a study they claimed, 'But good knowledge does not necessarily lead to good practice [18] but we think knowledge is very essential for preventing such a diseases which may be cause of an epidemic anywhere anytime. In our study we found, among total NS1 positive patients platelet count was <150 x109/L in 30.43% (n=49) and <100 x109/L in 49.69% (n=80) patients. Among 80 patients with platelet count <100 x109/L, 78 were with several grades of thrombocytopenia. In our study out of total 161 participants 22.98% had thrombocytopenia Grade 1 which was the highest portion. Then 14.29% had Grade 2, 7.45% had Grade 3 and 3.73% had Grade 4 thrombocytopenia. These findings may be helpful for the physicians as well as researchers in the treatment procedures and further researches.

Limitations of the study

This was a single centered study with a small sized sample. So the findings of this study may not reflect the exact scenario of the whole country.

CONCLUSION AND RECOMMENDATIONS

To prevent dengue fever awareness among people, government as well as the physicians is a must. The findings of this study may be helpful for physicians as well as for the researchers in further research on dengue fever. But for getting more specific information we would like to recommend for conducting more studies regarding the same issue with larger sample size.

REFERENCES

1. Ferreira GL. Global dengue epidemiology trends. Revista do Instituto de MedicinaTropical de São Paulo. 2012;54:5-6
2. Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ. Dengue: Acontinuing global threat. Nature Reviews. Microbiology. 2010;2460:7-14
3. Paranavitane S, Gomes L, Kamaladasa A, Adikari T, Wickramasinghe N, Jeewandara C. Dengue NS1 antigen as a marker of severe clinical disease. BMC Infect Dis. 2014;14 (1).
4. World Health Organization. Dengue: guidelines for diagnosis, treatment, prevention and control. Geneva: World Health Organization; 2009.
5. Guidelines on Management of Dengue Fever and Dengue Haemorrhagic Fever in Adults. National Guidelines. Revised and expanded edition 2012. Ministry of Health, Sri lanka. Available from http://www.epid.gov.lk/web/images/pdf/Publication/guidelines_for_the_management_of_df_and_dhf_in_adults.pdf. Accessed 29 January 2019.
6. Ralapanawa U, Alawattegama A, Gunrathne M, Tennakoon S, Kularatne S, Jayalath T. Value of peripheral blood count for dengue severity prediction. BMC Res Notes. 2018;11(1).
7. Dussart P, Petit L, Labeau B, Bremand L, Leduc A, Moua D et al. Evaluation of Two New Commercial Tests for the Diagnosis of Acute Dengue Virus Infection Using NS1 Antigen Detection in Human Serum. PLoS Negl Trop Dis. 2008;2(8):e280.
8. Brasier AR, Ju H, Garcia J, Spratt HM, Victor SS, Forshey BM, Halsey ES, Comach G, Sierra G, Blair PJ, Rocha C. A three-component biomarker panel for prediction of dengue hemorrhagic fever. The American journal of tropical medicine and hygiene. 2012 Feb 1;86(2):341-8.
9. Malhotra G, Yadav A, Dudeja P. Knowledge, awareness and practices of dengue in a city. International Journal of Medical Science and Public Health. 2014;3(3):295-299
10. Kularatne S, Gawarammana I, Kumarasiri P. Epidemiology, clinical features, laboratory investigations and early diagnosis of dengue fever in adults: a descriptive study in Sri Lanka. Southeast Asian J Tropl Med Public Health. 2005; 6(3):686-92.
11. Chaloemwong J, Tantiworawit A, Rattanathammethee T, Hantrakool S, Chai-Adisaksopha C, Rattarittamrong E, Norasetthada L. Useful clinical features and hematological parameters for the diagnosis of dengue infection in

patients with acute febrile illness: a retrospective study. BMC hematology. 2018 Dec;18(1):20.
12. Dengue haemorrhagic fever: Diagnosis, treatment, prevention,and contro. Geneva: World Health Organization; 1999.
13. Kulkarni RD, Patil SS, Ajantha GS, Upadhya AK, Kalabhavi AS, Shubhada RM, Shetty PC, Jain PA. Association of platelet count and serological markers of dengue infection-importance of NS1 antigen. Indian journal of medical microbiology. 2011 Oct 1;29(4):359.
14. Hottz ED, Lopes JF, Freitas C, Valls-de-Souza R, Oliveira MF, Bozza MT, Da Poian AT, Weyrich AS, Zimmerman GA, Bozza FA, Bozza PT. Platelets mediate increased endothelium permeability in dengue through NLRP3-inflammasome activation. Blood. 2013 Nov 14;122(20):3405-14.
15. Falconar A, Martinez F. The NS1 Glycoprotein Can Generate Dramatic Antibody-Enhanced Dengue Viral Replication in Normal Out-Bred Mice Resulting in Lethal Multi-Organ Disease. PLoS ONE. 2011;6(6):e21024.
16. Ho T, Wang S, Lin Y, Liu C. Clinical and laboratory predictive markers for acute dengue infection. J Biomed Sci. 2013;20(1):75
17. Hien TT, Vinh NN, Hien PT, Chinh NT, Simmons C, Wills B. Liver involvement associated with dengue infection in adults in Vietnam. The American journal of tropical medicine and hygiene. 2010 Oct 5;83(4):774-80.
18. Hairi F, Ong CH, Suhaimi A, Tsung TW, bin Anis Ahmad MA, Sundaraj C, Soe MM. Knowledge, attitude and practices (KAP) study on dengue among selected rural communities in the Kuala Kangsar district.