Journal of Infection and Public Health (2014) 7, 489—495

http://www.elsevier.com/locate/jiph

Dengue hemorrhagic fever: Comparison of patients with primary and secondary infections

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Received 10 February 2014; received in revised form 15 April 2014; accepted 24 May 2014

KEYWORDS
Dengue;
Dengue hemorrhagic fever;
Primary and secondary dengue infection;
Platelet count;
Alanine aminotransferase

Summary
Background: Dengue hemorrhagic fever (DHF) is considered to be associated with secondary dengue infection. This study was conducted to note frequency of primary and secondary dengue infection in DHF patients. Additionally these patients were compared in terms of age, gender, laboratory parameter, diseases severity and outcome.
Materials and methods: In this cross sectional observational study DHF patients fulfilling DHF criteria of Dengue Expert Advisory Group (DEAG) were included and divided into groups based on dengue specific IgG positivity and ratio of IgM to IgG. Group I, patients with secondary dengue infection were IgG positive or their ratio of IgM to IgG was <1.2. Group II, primary dengue infection patients were IgG negative or their ratio of IgM to IgG was >1.2. The two Groups were compared for statistically significant association in terms of age, gender, laboratory parameter (at admission hematocrit [HCT], platelet, white blood cell [WBC] counts, alanine aminotransferase [ALT] value), severity (DHF or dengue shock syndrome), and outcome (recovered or expired).
Results: Two hundred thirty-four DHF patients were included. 66.2% was male and 33.8% female. Mean patient age was 28.8 ± 12.4 years. Based on dengue markers results, 61.5% patients were categorized to Group I, and 38.5% to Group II. Statistically significant association between the two Groups was noted in terms of at admission platelet count, and ALT value, P value <0.05.

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http://dx.doi.org/10.1016/j.jiph.2014.05.005

Conclusion: Primary dengue infection is frequently associated with DHF. Patients with DHF caused by secondary dengue infection have lower at admission platelet counts and higher ALT value.

Introduction

Dengue is arthropod-borne viral illness caused by infection with one of the 4 serotypes of dengue virus. Dengue viruses are RNA viruses that belong to the Flavivirus genus. Dengue is an important global health care issue. At least 3.6 billion people living in more than 125 tropical and subtropical countries are at risk of developing dengue infection [1]. Internationally, up to 22,000 deaths are attributed to dengue per year [2].

Dengue infection can result in dengue fever (DF) and dengue hemorrhagic fever (DHF). The latter is further divided into four grades depending upon severity [3,4]. Infection with one of the dengue viruses confers lifelong immunity to that serotype. If a person is infected by another serotype of dengue virus (secondary infection), problematic versions of dengue, such as dengue hemorrhagic fever, may develop due to immune enhancement [4—6]. DHF can be associated with poor outcomes depending on the facilities available for patient management [3,4].

The detections of the NS1 antigen and dengue-related IgM and IgG antibodies are commonly used in dengue diagnoses [4,7]. NS1 is virus-specific non-structural protein that can be detected for up to 9 days after dengue infection [4]. IgM antibodies become detectable in most patients at days 3—5 of fever onset. Tests for NS1 and IgM antibodies have high sensitivities (~90%) and specificities (98%) in the diagnosis of dengue at ≥5 days from fever onset. IgG dengue-specific antibodies are used to diagnose prior dengue infections [4]. Various characteristics of patients with dengue infections have been the focus of extensive research. However, there is a paucity of studies that have focused on DHF patients and have emphasized primary and secondary infections.

The first outbreak of dengue in Pakistan occurred in 1994—1995. Since that time, dengue has increasingly been recognized as an important health care issue [8—10]. A dengue epidemic occurred in Rawalpindi, Pakistan from July to December of 2013 [11,12]. In this epidemic, 1175 patients suffering from dengue infections were diagnosed at public sector hospitals. Of the 811 patients who were managed at Holy Family Hospital, Rawalpindi, 255 had DHF. This study sought to note the frequencies of primary and secondary infections in DHF patients. Additionally, age, gender, severity of DHF, hematological parameters at admission (i.e., hematocrit [HCT] and platelet and white blood cell [WBC] counts), alanine aminotransferase (ALT) level, duration of hospital stay, and outcome were compared between patients with primary and secondary dengue infections.

Materials and methods

Study and participants

This cross sectional, observational study was conducted at the Medical Unit of the Holy Family Hospital, Rawalpindi from the 1st of July 2013 to the 31st of December, 2013. All patients with dengue infection who fulfilled the DHF diagnostic criteria and were managed at this hospital were evaluated. Patients with illnesses associated with hematological abnormalities, ascites and/or pleural effusion were excluded. These exclusion criteria included chronic liver disease, autoimmune disorders, hematological disorders, renal failure, etc. Patients with unknown dengue IgG statuses were also excluded from the study. The patients were managed in a standard manner as per the Dengue Expert Advisory Group (DEAG) protocol for DHF/dengue shock syndrome (DSS) [13].

Ethical considerations

This study was approved by the Departmental Ethical Committee, and the patients were included after they or their surrogates provided informed consent.

Dengue diagnostic criteria

Dengue infection: The diagnosis of dengue infection was based on the DEAG criteria [13].

DHF: DHF is characterized by plasma leakage revealed by hemoconcentration (an increase in hematocrit ≥20% above the age-adjusted average or a decrease in hematocrit ≥20% of the baseline following fluid replacement therapy), pleural

effusion, ascites, or hypoproteinemia plus one of the following: febrile illness of 2—7 days duration, hemorrhagic manifestations or a positive tourniquet test, and thrombocytopenia $\leq$100,000/mm^3 [13,14].

DSS: DSS is severe version of DHF. DSS exhibited all of the features of DHF and circulatory failure evidenced by a rapid, weak pulse, a low pulse pressure (20 mmHg) or age-specific hypotension and cold, clammy skin, and restlessness [13—15].

Primary and secondary dengue infections: Each patient's serological status pertaining to dengue infection was determined based on NS1 and dengue-specific IgM and IgG positive or negative results and titers. These tests were performed using the SD Dengue Capture ELISA Kit for dengue NS1, IgM and IgG. This kit has sensitivities and specificities, respectively, of 92.7% and 98.4% for NS1, 96.4% and 98.9% for IgM, and 98.8% and 99.2% for IgG. Based on IgG positivity and the ratio of IgM to IgG, the patients were categorized into secondary (Group I) and primary (Group II) dengue infection groups. Group I patients were NS1- and/or IgM-positive [16]. Additionally, these patients were IgG-positive or had ratios of IgM to IgG <1.2 [7,16—18]. The Group II patients were NS1- and/or IgM-positive and were IgG-negative or exhibited ratios of IgM to IgG >1.2 when they were IgG-positive [4,7,17,18].

Data and statistical analysis

Group, age, gender (male, female), duration of stay, outcome (discharged or expired), and HCT, WBC, platelet count, and ALT value at admission for each patient were noted on a specifically designed pro forma. For each group, the frequencies and percentages were calculated for the qualitative variables, and the means $\pm$ the SDs were calculated for the quantitative variables. Statistically significant associations between the two Groups were identified with *t*-tests, Chi-squared tests, or Fisher's exact tests, as appropriate. Sub-analyses of the secondary and primary DHF patients in terms of severity (i.e., DHF or DSS) were also performed.

Results

Two hundred fifty-five (255) patients with DHF were identified. The dengue IgG statuses of some of the patients were not known, and these patients were excluded. Ultimately, 234 patients were included in the study, Fig. 1 provided details about the included patients. One hundred fifty-five (66.2%) patients were male, and 79 (33.8%) were female. The mean patient age was 28.8 $\pm$ 12.4 years. Two hundred twenty-three (95.3%) patients were diagnosed with DHF, and 11 (4.7%) were diagnosed with DSS. Two hundred twelve (90.6%) patients recovered and were discharged, 8 (3.4%) expired, 9 (3.8%) were discharged on request, and 5 (2.1%) left against medical advice.

Based on the dengue marker results, 61.5% of the patients were categorized as Group I, and 38.4% were categorized as Group II (Fig. 1). The ages, genders, severity-based dengue diagnoses (i.e., DHF or DSS), durations of hospital stays, outcome-based analysis and hematocrit, platelet, and WBC counts at admission for the two groups are given in Table 1. Statistically significant associations were noted between the Group I and II patients in terms of platelet count and ALT value at admission. Regarding disease severity, 95.1% of the Group I patients had DHF, and 4.9% of these patients had DSS. Similarly, 95.5% of the Group II patients had DHF, and 4.5% of these patients had DSS. Detailed comparisons of the disease severities of the Group I and II patients are provided in Tables 2 and 3, respectively.

Discussion

An exaggerated immune response or an immune enhancement is thought to be an important pathophysiological basis of the development DHF and DSS, which are severe versions of dengue infection [5,6]. The antibodies that develop as a result of dengue infection confer immunity against that particular dengue virus type but are non-neutralizing for infections caused by other dengue virus serotypes. Such infections by alternate dengue virus serotypes combined with cross-reactive T cells are believed to cause most of the manifestations of DHF/DSS [5,6]. Secondary infections are thus more frequently associated with DHF/DSS. The majority of our patients had secondary dengue infections.

DHF rarely results from primary dengue infection [19,20]. However, our findings in this regard are unusual in that a greater proportion of the DHF patients (36.3%) had primary dengue infections. Co-infection with more than one viral serotype and unknown viral and host factors seem to be responsible for this observation [21,5,22—25]. However, an appropriate explanation for our results cannot be provided due to the limitations of this study; i.e., the sole reliance on IgG positivity and/or the IgM:IgG ratio as a marker of secondary dengue infection, the timing of the

Table 1 Comparison of patients with primary (Group II) and secondary (Group I) dengue infection.

	Group I (144/234)	95%CI[a]	Group II (90/234)	95%CI[a]	*P* value
Age	29 ± 13	±2.14	28.5 ± 11.4	±2.36	0.76
Gender					
Male	96 (66%)	±7.74	59 (65.55%)	±9.82	0.43
Female	48 (33%)	±7.68	31 (34.44%)	±9.82	
Diagnosis					
DHF	137 (95.13%)	±3.52	86 (95.55%)	±4.28	0.45
DSS	7 (4.86%)	±3.51	4 (4.44%)	±4.26	
Hematocrit[b] (%)	40.4 ± 5.4	±1.83	41.1 ± 4.6	±2.77	0.60
Platelets[b] (/mm^3)	37,286.9 ± 24,026.4	±3937.94	54,813.3 ± 34,471.8	±7121.82	0.00004
WBC[b] (/mm^3)	4.8 ± 2.9	±0.48	4.4 ± 2.4	±0.51	0.25
ALT[b] (Units/L)	160.1 ± 177.1	±35.26	99.7 ± 85.6	±22.24	0.0005
Duration of stay (days)	4.3 ± 1.6	±0.27	4.4 ± 1.8	±0.4	0.66
Outcome[c]					
Expired	5 (3.7%)	±3.08	3 (3.52%)	±3.81	0.48
Recovered	130 (96.29%)	±3.09	82 (96.4%)	±3.85	

[a] Confidence interval.
[b] At admission.
[c] Patients who were discharged on request or left against medical advice were not included.

Table 2 Secondary dengue infection patients: DHF/DSS wise comparison.

	DHF (137/144)	95%CI[a]	DSS (7/144)	95%CI[a]	*P* value
Age	28.9 ± 13.1	±2.21	31.3 ± 10.5	±8.45	0.757
Gender					
Male	89 (64.9%)	±7.8	7 (100%)	±0000	0.05
Female	48 (35.1%)	±7.8	0 (0%)	±0000	
Hematocrit[b] (%)	40.4 ± 5.4	±1.93	43.4 ± 5.7	±5.64	0.21
Platelets[b] (/mm^3)	36,581 ± 22,504	±3782.14	51,000 ± 45,125	±33,428.94	0.43
WBC[b] (/mm^3)	4.7 ± 2.8	±0.48	5.8 ± 3.9	±2.88	0.48
ALT[b] (Units/L)	152.4 ± 169	±34.93	259.1 ± 256.7	±191.7	0.07
Duration of stay (days)	4.3 ± 1.2	±0.21	5.1 ± 5.1	±3.81	0.0000001
Outcome[c]					
Expired	0 (0%)	±0000	5 (71.4%)	±33.48	0.0000001
Recovered	128 (100%)	±0000	2 (28.6%)	±33.48	

[a] Confidence interval.
[b] At admission.
[c] Patients who were discharged on request or left against medical advice were not included.

Table 3 Primary dengue infection patients: DHF/DSS wise comparison.

	DHF (86/90)	95%CI[a]	DSS (4/90)	95%CI[a]	*P* value
Age	28.2 ± 11.1	±2.36	35.5 ± 16.5	±16.21	0.18
Gender					
Male	56 (62.2%)	±10.02	3 (75%)	±42.43	0.9
Female	30 (37.8%)	±10.02	1 (25%)	±42.43	
Hematocrit[b] (%)	41.1 ± 4.6	±2.19	40.9 ± 18.4	±25.58	0.98
Platelets[b] (/mm^3)	54,409 ± 33,526	±7085.77	63,500 ± 57,448	±56,298.4	0.7
WBC[b] (/mm^3)	4.2 ± 2.2	±0.5	8.3 ± 4.1	±4.03	0.03
ALT[b] (Units/L)	101.3 ± 87.1	±23.26	71.6 ± 52.3	±59.19	0.43
Duration of stay (days)	4.3 ± 1.5	±0.33	6.5 ± 5.8	±5.68	0.000000134
Outcome[c]					
Expired	0 (0%)	±0000	3 (75%)	±42.43	0.00002
Recovered	81 (100%)	±0000	1 (25%)	±42.43	

[a] Confidence interval.
[b] At admission.
[c] Patients who were discharged on request or left against medical advice were not included.

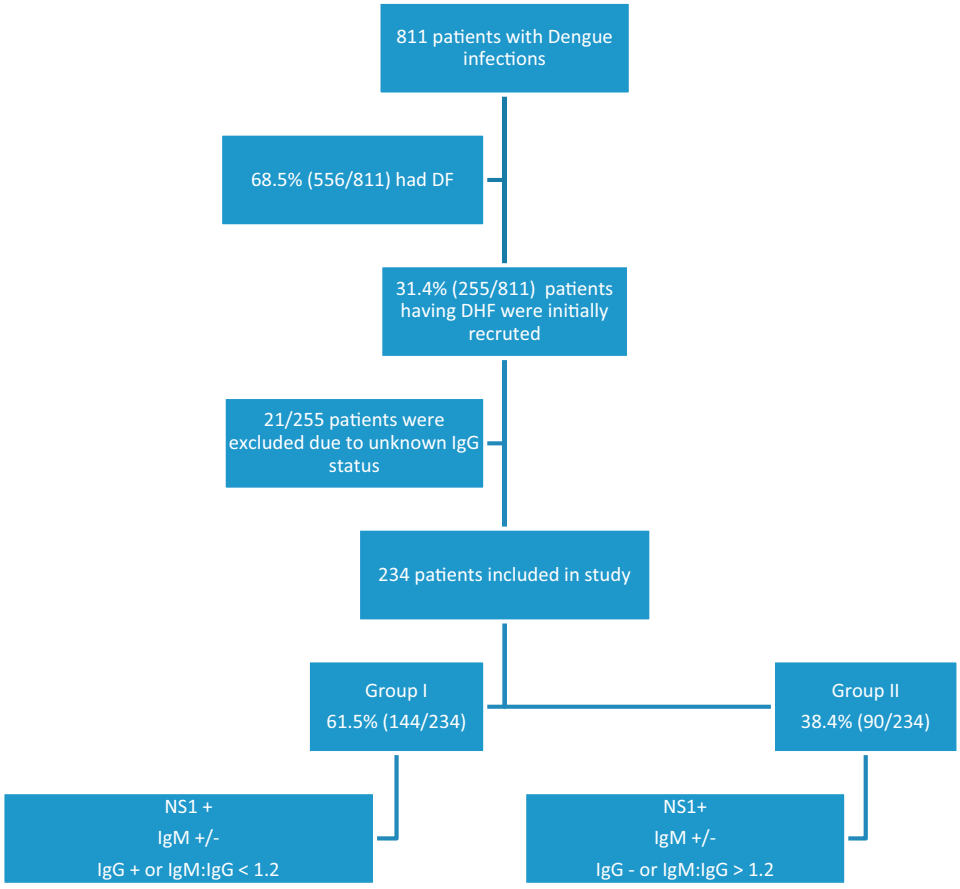

Figure 1 Flow diagram showing patients and Groups.

sampling, and the acquisition of single samples for serodiagnosis.

We did not observe a difference in the severity of DHF between the patients with primary and secondary infections. A sub-group analysis in a study conducted in Thailand revealed that 18.8% of patients with primary infections and 42% of the patients with secondary infection developed severe forms of dengue [26]. Another study reported that 77.7% of primary and 94.4% of secondary dengue infection patients had DHF. In the same study, 17.3% of patients with primary infection were found to have DSS, and 50.4% of the patients with secondary infections had DSS [25]. Another study reported that DSS was observed only in patients with secondary dengue infections [15].

The mortality of DHF varies by location depending on expertise of the management teams and the severity of illness at the time of admission [27]. DHF/DSS-related mortality is 2—44% [27]. In a Malaysian study that reviewing the mortality of DHF, 100% of DHF mortalities were attributed to secondary dengue infections [17]. A Pakistani study reported that 75% of DSS-related mortalities were due to secondary dengue infections [16]. No significant difference between the mortalities of the DHF patients with primary and secondary infections was observed in our study.

We did not observe significant differences between the primary and secondary DHF patients in terms of age, gender, disease severity, duration of stay, outcome, or hematocrit or WBC counts at admission. However, the patients with secondary DHF exhibited significantly reduced platelet counts and elevated higher ALT values at admission, and these results are indicative of severe disease [17]. Interestingly, a sub-analysis of the illness severities (i.e., DHF or DSS) of our primary and secondary infection patients revealed significant differences in the durations of hospital stays and outcomes. Additionally, the initial platelet counts of the primary dengue infection patients differed significantly based on the diagnosis of DHF or DSS.

The goal of dengue management is to stabilize hemodynamic the statuses of DHF/DSS patients to maintain perfusion of the vital organs during the critical phase. Predicting the development of DHF

or DSS in a particular patient at the onset of dengue infection is difficult. Patients with dengue infection who exhibit warning signs and/or clinical features that are suggestive of the onset of DHF are hospitalized. This situation is costly for socioeconomically disadvantaged facilities, such as our own, in which other health care resources must be diverted for the management of dengue patients. This diversion of resources can potentially increase the suffering of patients with other illnesses. It is possible that the recognition of secondary dengue infection and the focused management of these patients might improve outcomes [4,16]. The important implication of findings is that prompt and efficient management is the key to successful treatment of dengue-infected patients. The erroneous belief that primary dengue infection rarely causes DHF may prove to be problematic.

Conclusion

DHF is frequently caused by primary dengue infection. The DHF patients with secondary dengue infection exhibited lower platelet counts and ALT values at admission. The DHF patients with primary and secondary infections did not significantly differ in terms of age, gender, disease severity, duration of stay, outcome or HCT or WBC count at admission.

Funding

No funding sources.

Competing interests

None declared.

Ethical approval

Not required.

Acknowledgments

We are indebted to the Dengue Team and the administration and staff of the Medical & Allied Departments of the Holy Family Hospital, Rawalpindi for their support in the completion of this study.

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