



ORIGINAL ARTICLE

Association of pattern of thrombocytopenia and serology with timings of plasma leakage in patients of dengue hemorrhagic fever during dengue epidemic 2019 – an experience from Rawalpindi Medical University: A cross sectional study.

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ABSTRACT... Objective: To find the association between the patterns of thrombocytopenia with the timings and patterns of plasma leakage in patients presenting with dengue hemorrhagic fever (DHF) during the dengue epidemic of 2019, in Rawalpindi city. **Study Design:** Cross-sectional study. **Setting:** Department of Medicine, District Headquarter Hospital, Rawalpindi, Pakistan. **Period:** August to November 2019. **Material & Methods:** In which patients who were labelled and managed as DHF on the basis of clinical presentation, positive serology and ultrasonic evidence of plasma leakage were included. Demographic profile, clinical features with duration of illness, laboratory investigations including serological markers (NS1, IgM and IgG) and ultrasonic findings were recorded on a self-structured performa and data was analyzed using SPSS v23.0. **Results:** Two hundred and ninety five patients with DHF were enrolled in the study. There were 2012 males and 83 females (male to female ratio of 2.5:1). Mean age of all participants was 32.83 years. 50.5% of the cases were primary infection (based on serology), whereas 38.3% were secondary infection and 11.2% were triple negative infection. In 81.1% of the cases, plasma leakage occurred on the 4th to 7th day of illness. In these cases, platelet count was <40,000 cells/mm³ in 13.6% cases, between 40,000 cells/mm³ - 80,000 cells/mm³ in 50.8% cases and greater than 80,000 cells/mm³ in 35.5% cases. Amongst the 17.8% of cases where plasma leakage occurred between 8th to 10th days, 56.6% had platelet count greater than 80,000 cells/mm³, 22.6% has platelet count between 40,000 cells/mm³- 80,000 cells/mm³ and 20.8% had platelet count of less than 40,000 cells/mm³. **Conclusion:** Most people had plasma leakage at day 4-7 of illness, so needed more critical monitoring and admission at 4th to 7th day of illness, plasma leakage was observed more commonly in primary as compared to secondary infections and mostly at the day of leakage, platelet count was between 40,000 cells/mm³- 80,000 cells/mm³. Strict monitoring is required from the 4th day of illness to detect early signs of leakage to decrease the mortality and complications by dengue infection.

Key words: Dengue Hemorrhagic Fever, Plasma Leakage, Thrombocytopenia.

INTRODUCTION

Dengue is an arbovirus infection, endemic to the tropical and subtropical areas around the world.¹ This infection commonly causes dengue fever (DF) and dengue hemorrhagic fever (DHF). According to WHO, nearly 100 million cases of dengue fever and around 250,000-500,000 cases of dengue hemorrhagic fever occur annually.^{2,3} Dengue fever and dengue hemorrhagic fever can be caused by any of the 4 serotypes of the dengue viruses (DENV-1, -2, -3, or -4).⁴ All form of these dengue viruses can be found in areas infested

with dengue vectors (mainly *Aedes aegypti*).⁵

The defining feature of severe dengue infection is the alteration in the vascular permeability, which causes plasma leakage (DHF); this can ultimately lead to hypovolemic shock causing dengue shock syndrome.⁶ This increased vascular permeability is thought to be due to endothelial activation/dysfunction, which results from decreased nitric oxide release, leading to impaired vascular relaxation.⁷ Clinically, dengue hemorrhagic fever is a febrile illness which presents as plasma

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leakage (manifested by hemoconcentration, ascites and pleural effusion), bleeding tendency and liver involvement; some cases can result in hypovolemic shock and dengue shock syndrome.^{8,9}

Monitoring for the clinical signs of plasma leakage is imperative in dengue hemorrhagic fever, in order to effectively prevent the progression of the illness to shock syndrome. Plasma leakage is monitored to give adequate fluid therapy.^{10,11} Moreover, thrombocytopenia is also present in dengue hemorrhagic fever, as it is one of the diagnostic criteria for DHF. The aim of our study was to find the association between the patterns of thrombocytopenia with the timings and patterns of plasma leakage in patients presenting with dengue hemorrhagic fever during dengue epidemic 2019.

MATERIAL & METHODS

This retrospective study was conducted at the department of Medicine, District headquarters hospital, Rawalpindi, from August 2019 to November 2019. A total of 295 serum samples were collected from clinically suspected cases of dengue hemorrhagic fever (DHF), using consecutive sampling, for the cross sectional study of the variables.

The study participants were included on the basis of fulfillment of the WHO criteria for DHF. DHF cases were defined as patients as having a dengue infection and satisfying the following criteria: rise in Hct of 20% from baselines, or fall of Hct greater than 20% after fluids/blood transfusion, plasma leakage evidence such as ultrasound chest showing pleural effusion/ascites/ pelvic fluids/ gallbladder wall thickness or positive tourniquet test result, and platelet count of less than or equal to 100,000/mm³.^{12,13,14} On the other hand, patients with any of the following conditions were excluded from the study: history of concomitant illness/infection such as typhoid infection, acquired immunodeficiency syndrome, any hematological/bleeding disorder or other known causes of acute febrile syndromes. The DHF cases were labelled primary infection, secondary infection or triple negative infection,

on the basis of presence/absence of serological markers (NS1, IgM, and IgG). These serological markers were identified in the serum using the ELISA kit. Primary infection was defined as the presence of NS1 antigen alone, IgM antigen alone or the presence of both NS1 and IgM. On the other hand, for a sample to be labelled as a secondary infection, following dengue parameters had to be positive: (1) IgG only (2) NS1 and IgG (3) IgM and IgG (4) NS1, IgG and IgM. The cases where all three serological markers were absent were termed as 'triple negative infection'

The study protocol was reviewed and approved by the ethical committee of Rawalpindi Medical University, Rawalpindi. Moreover, informed consent was obtained from the participants before taking their sample for the study, and the patient confidentiality was maintained.

The data for the 295 participants was recorded using a self-structured performa. The performa recorded the demographic profile, clinical features with duration of illness, laboratory investigations including serological tests (NS1, IgM, and IgG) and ultrasonic findings of all patients. The blood parameters (including the platelet count, hematocrit, TLC and hemoglobin) was also recorded at the day of plasma leakage. This data was then analyzed using SPSS v23.0, which compared the results by applying Chi-square test on different variables.

RESULTS

A total of 295 samples were collected from suspected dengue hemorrhagic fever (DHF) cases. Different variables were then recorded from these 295 samples [Table-I]. After that, we analyzed the association between the day of plasma leakage with the different parameters (gender, age, platelet count and type of infection based on serology) amongst the DHF patients [Table-II].

Of the 295 samples, 212 (71.4%) samples belonged to males, whilst 83 (27.9%) were from females. The mean age of the sample population was 32.83 ± 13.08 years. The samples were tested for presence of 3 serological markers:

NS1 antigen, IgM antibody and IgG antibody. Primary infection was defined as the presence of NS1 antigen alone, IgM antigen alone or the presence of both NS1 and IgM. In our study, 149 out of 295 cases (50.5%) were classified as primary infection. On the other hand, for a sample to be labelled as a secondary infection, following dengue parameters had to be positive: 1) IgG only 2) NS1 and IgG 3) IgM and IgG 4) NS1, IgG and IgM. The number of secondary infection cases was 115 (38.3%) in our study. The cases where all three serological markers were absent were termed as 'triple negative infection'; for our study, there were 33 (11.2%) triple negative infection cases.

The day when plasma leakage started to occur for each of the 295 cases was also recorded. The data was then classified into either plasma leakage on 4-7th day of illness or plasma leakage on 8-10th day of illness. Among 241 out of 295 (81.1%) cases, the day of plasma leakage was in between 4-7th day of illness, whereas, in 53 (17.8%) cases, plasma leakage occurred between 8-10th day of illness. From the blood parameters that were recorded on the day of leakage, the mean platelet count on the day of leakage was 80.6 ± 51.2 .

On comparing the effects of gender on the day of plasma leakage, the difference was found to be significant (P-value = 0.008). In the sample study, 68.6% males and 31.4% females had plasma leakage in between 4-7th day of illness. This is in comparison to 86.8% males and 13.2% females with plasma leakage occurring on the 8-10th day of illness.

The effect of age on the day on plasma leakage was analyzed by classifying the data into 2 groups: Less than 35 years old and 35 years or more. The difference of day of plasma leakage due to age group was found to be insignificant (p-value = 0.137).

The platelet count recorded on the day of plasma leakage was either less than 40,000 cells/mm³, between 40,000 cells/mm³ to 80,000 cells/mm³ or greater than 80,000 cells/mm³. In the cases

where plasma leakage occurred on 4-7th day, the platelet count was less than 40,000 cells/mm³ in 13.6% of the cases, between 40,000 cells/mm³ to 80,000 cells/mm³ in 50.8% cases and greater than 80,000 cells/mm³ in 35.5% of the cases. This is in contrast to the cases where plasma leakage occurred on 8-10th day, as amongst those, 56.6% cases had platelet count greater than 80,000 cells/mm³, and only 20.8% and 22.6% cases had platelet count less than 40,000 cells/mm³ and in between 40,000 cells/mm³- 80,000 cells/mm³, respectively. This association of plasma leakage with platelet count was found to be significant, with p-value of 0.001.

To analyze the association between the day of plasma leakage and the type of infection (based on serology), the sample study was classified into primary, secondary and triple negative infection. Amongst the cases where plasma leakage occurred between 4-7th day of illness, 52.5% were primary infection, 37.2% were secondary infection and 10.3% were triple negative infections. On the other hand, the percentage of primary and secondary infection cases with plasma leakage on 8-10th day was 43.4% each; whilst 13.2% cases were triple negative infections. This difference was found to be extremely significant with p-value of 0.000.

Variable	N=295
Gender	
Male	212 (71.4%)
Female	83 (27.9%)
AGE	32.82 ± 13.08 years
Serology Markers	
NS1 Ag	70 (23.7%)
IgM Ab	10 (3.4%)
IgG Ab	10 (3.4%)
NS1 Ag + IgM Ab	62 (21%)
NS1 Ag + IgG Ab	33 (11.2%)
IgM Ab + IgG Ab	18 (6.1%)
NS1 Ag + IgM Ab + IgG Ab	59 (20%)
Triple Negative Infection	33(11.2)
YPE of Infection Based on Serological Markers	
Primary	149 (50.5%)
Secondary	115 (38.3%)
Triple Negative	33 (11.2%)
Day of Plasma Leakage	
4-7 th DAY OF ILLNESS	241 (81.1%)
8-10 th DAY OF ILLNESS	53 (17.8%)
Blood Parameters at Day of Leakage	
Hemoglobin (g/dl)	13.8 ± 2.11
TLC (cells/mm ³)	4.79 ± 2.46
Platelet count (cells/mm ³)	80.6 ± 51.2
Hematocrit (%)	39.7 ± 5.19

Table-I. Descriptive statistics for DHF (dengue hemorrhagic fever) patients (N=295)

Groups	Day of Plasma Leakage		Total (N= 295) (100%)	P-Value*
	4-7 TH Day (N = 242) (82%)	8-10 TH Day (N = 53)(18%)		
Gender				
Male	166 (68.6%)	46 (86.8%)	212 (71.2%)	0.008
Female	76 (31.4%)	7 (13.2%)	83 (28.1%)	
Age Groups				
Less Than 35 Years	150 (62.0%)	27 (51.0%)	177 (60.0%)	0.137
35 Years or More	92 (38.0%)	26 (49.0%)	118 (40.0%)	
Platelet Count at Day of Leakage				
Less Than 40,000	33 (13.6%)	11 (20.8%)	44 (14.9%)	0.001
40,000 – 80,000	123 (50.8%)	12 (22.6%)	135 (45.8%)	
Greater Than 80,000	86 (35.5%)	30 (56.6%)	116 (39.3%)	
Type of Infection Based on Serology				
Primary	127 (52.5%)	23 (43.4%)	150 (50.8%)	0.000
Secondary	90 (37.2%)	23 (43.4%)	113 (38.3%)	
Triple Negative Infection	25 (10.3%)	7 (13.2%)	32 (10.8%)	

Table-II. Association of day of plasma leakage with gender, age, platelet count and type of infection based on serological markers
*P-value less than 0.05 was considered significant.

DISCUSSION

This study supports the association between plasma leakage, which is the hallmark of DHF, with variation in gender, platelet count and the types of infection (based on serology). In our study, the mean age group of the sample population was 32.82 ± 13.08 years, comprising of 71.4% males and 27.9% females. A similar study conducted by Riffat Mehmoob et al. at Pathology Department of King Edward Medical University, Lahore included the following age groups: age group of 16-30 years (48%), 24% in age group of 31-45 years, 24% in 46-60 years, 2% in 61-75 years age group and above, and no patient in the age group 1-15 years. The sample included 44% males and 56% females in this specific study.¹⁵ Another similar study conducted by Subhash C Arya et al. included population with mean age of 31.2 years; this makes it very closely related to the study we conducted.¹⁶ The variation of day of plasma leakage across gender was significant, whilst the variation across age groups was insignificant. Overall, greater percentage of male population had plasma leakage on both days 4-7 of illness and days 8-10 of illness and less females were effected overall. This can be due to the fact that according to cultural norms amongst the study population, males are assigned the role to take care of business or having to go to mosque to

pray which involves leaving the house, hence are more likely to get bitten by the mosquitoes when outdoor. Moreover, due to the weather conditions at the time, men tend to be dressed in clothes which may not cover skin fully; but due to religious and cultural reasons, this is not the case for females. Plasma leakage occurred more on day 4-7 of illness amongst men, and hence, it is imperative to monitor patients vigilantly on these days.

Although, the gold standard for the diagnosis of acute dengue infection is virus isolation and characterization, the technique is rather expensive and time consuming; moreover, the facilities that are required for the process are mostly unavailable at hospital laboratory facilities.¹⁷ Most common serological markers that are used to detect and classify the infection into primary, secondary or triple negative dengue infection are NS1 antigen, IgM antibody and IgG antibody. Following was the frequency of presence of each serological marker in our sample population: NS1 Ag alone (23.7%), IgM Ab alone (3.4%), IgG Ab alone (3.4%), NS1 Ag + IgM Ab (21%), NS1 Ag + IgG Ab (11.2%), IgM Ab + IgG Ab (6.1%), NS1 Ag + IgM Ab + IgG Ab (20%) and no serological marker present in 11.2% of the sample. These values are comparable with the results gathered

from research conducted by Subhash C Arya et al. where the frequency of each serological marker was as follows: NS1 Ag alone (20.9%), IgM Ab alone (2.55%), IgG alone (5.09%), NS1 Ag + IgM Ab (7.69%), NS1 Ag + IgG Ab (1.54%), IgM Ab + IgG Ab (6.47%), NS1 Ag + IgM Ab + IgG Ab (5.83%) and triple negative infections (49.9%).¹⁶ The drastic difference in the number of triple negative cases between the two studies may be due to presence of another acute febrile illness in the region where the study conducted by Subhash C Arya et al. took place.

Upon looking at the correlation between day of plasma leakage and the type of infection based on serology, it was noticed that in the cases where the plasma leakage occurred between the days 4 to day 7 of illness, the maximum cases were of primary infection (52.5%), followed by secondary infection (37.2%) and then triple negative infection (10.3%). The triple negative infections should be investigated further for acute febrile illnesses including malaria, urinary tract infection, enteric fever, Chikungunya virus and the influenza virus H1N1 infection, to rule out the possibility of the case being something other than dengue hemorrhagic fever.¹⁶ In the cases where the plasma leakage occurred on day 8th to day 10th of illness, 43.4% were primary infection, 43.4% were secondary infections and 13.2% were triple negative infection. This shows that cases of primary infection decreased as the plasma leakage occurred late in the illness (between days 8-10). On the other hand, the cases of secondary infection were more when the plasma leakage occurred between days 8-10 of illness, in comparison to when plasma leakage happened earlier in the illness.

The mean hemoglobin level in our study sample was 13.8 ± 2.11 , whereas the mean total leukocyte count was 4.79 ± 2.46 , mean hematocrit was 39.7 ± 5.19 and mean platelet count was found to be 80.6 ± 51.2 . In the study conducted by Subhash C Arya et al, the mean platelet count was recorded for NS1 positive and NS1 negative patients separately, and was 116.8 ± 70.2 and 167.2 ± 94.0 , respectively.¹⁷ The variation in the platelet counts of the patients across the studies

is understandable, as the mechanism of dengue related thrombocytopenia and coagulopathy requires platelet activation, pro-coagulant and anti-coagulant arm of the coagulation system, complement system, cytokines and endothelial cells; hence, variations can occur.¹⁶

In the cases where the plasma leakage occurred in between day 4-7 of illness, majority of the samples were recorded to have a platelet count of 40,000 cells/mm³ - 80,000 cells/mm³. This is in contrast to plasma leakage at day 8-10 of illness, where majority of the patients had platelet count greater than 80,000 cells/mm³. This further reinforces the need to vigilantly monitor the blood parameters and detect early signs of leakage around 4-7 day of illness.

CONCLUSION






Most people had plasma leakage at day 4-7 of illness, so needed more critical monitoring and admission at 4th to 7th day of illness, plasma leakage was observed more commonly in primary as compared to secondary infections and mostly at the day of leakage, platelet count was between 40,000 cells/mm³- 80,000 cells/mm³. Strict monitoring is required from the 4th day of illness to detect early signs of leakage to decrease the mortality and complications by dengue infection.
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AUTHORSHIP AND CONTRIBUTION DECLARATION

No.	Author(s) Full Name	Contribution to the paper	Author(s) Signature
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2	Nimra Waheed	Article writing.	
3	Shehzad Manzoor	Correction or review.	
4	Shamaila Mumtaz	Ethical board review.	
5	Hamza Waqar Bhatti	Proof reading.	
6	Tahira Yasmeen	Data analysis.	