

Association of clinical features and hematological parameters with serotype-specific dengue virus infection

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Objective: To identify the relationship of common serotypes of dengue virus in our region with hematological changes in dengue patients.

Methodology: This observational study was conducted at Isra University Hospital, Hyderabad and included 100 cases of dengue fever. Dengue virus antibodies were confirmed by using dengue virus rapid test. Serotypes of virus were confirmed by immunofluorescence test. Hematocrit (HCT), total leukocyte count (TLC) and platelet count were determined.

Results: The association of majority of common clinical presentation with dengue serotypes was found as non-significant except bleeding, which was found

significant. The association of TLC was found significantly associated with severity of serotype. There were only two cases of serotype III and both cases were found with decreased TLC. Thrombocytopenia was significantly associated with serotype. However no significant difference was found between hematocrit values and serotype of dengue virus.

Conclusion: Bleeding was only clinical presentation associated significantly with dengue serotypes. The low platelets count and TLC were also significantly associated with severity of dengue serotype. Patients with serotype III had severe thrombocytopenia.

Keywords: Dengue virus, serotype, thrombocytopenia, total leukocytes count.

INTRODUCTION

Dengue virus (DENV) is a positive sense, single stranded RNA enveloped virus that comprises of four serotypes (DENV 1, 2, 3 and 4).¹ Infection occurs due to the bite of the mosquito.² About 100 countries are declared as endemic for dengue fever by WHO.³ The annual infection rates are nearly 50 to 100 million and among these approximately 500,000 require hospitalization every year.^{4,5} The fatality is common in children and young adults and reaches up to 5% every year.⁶ Two outbreaks of dengue have been reported in Pakistan in year 1994 and 2005.⁷

Serotypes type 2, 3 and 4 are seen in Pakistan.^{8, 9} All four serotypes are serologically related, but antigenically distinct.¹⁰ They produce a spectrum of clinical illnesses ranging from a classical dengue fever (DF) to severe and potentially fatal complications known as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).¹¹ Dengue fever is marked by a sudden onset of high grade fever, severe headache and retro-ocular pain and myalgia. The distinctive characteristics of DHF and DSS consist of hemorrhagic manifestations, plasma leakage, and profound shock.^{12,13}

In 2011, dengue outbreak was reported from Lahore, Pakistan and according to Health Department, more than 23,000 people were hospitalized, of which at least 365 people died due to DHF and DSS. DENV-2 and DENV-

3 and a single case of DENV-4 were reported in the 2011 dengue outbreak.¹⁴ This present study was carried out to identify the relationship of common serotypes of dengue virus in our region with hematological changes in these patients.

METHODOLOGY

This observational study was conducted at Isra University Hospital, Hyderabad and Liaquat University of Medical and Health Sciences during July to December 2015. The patients were selected through convenience sampling and 100 patients of suspected dengue fever were included, which were calculated through the formula for sampling for proportions. The patients already suffering from hemorrhagic diathesis due to other causes, P.U.O, patients on medication including steroids and patients with viral infections other than dengue were excluded from the study.

About 2.5 ml in Gel tube for serotyping and 3ml blood in EDTA tube was collected for hematocrit (HCT), platelets count and total leukocyte count (TLC), which were performed on automated hematology analyzer (Sysmex XN550). Dengue virus antibodies were confirmed by using dengue virus rapid test. Serotypes were confirmed by immunofluorescence test, antibodies of virus were stained with fluorescein labeled anti-human antibodies and antigen antibody complex

appeared coarse granular structures under Fluorescence Microscope.

Statistical Analysis: The data were analyzed using SPSS version 21. The tests of significance were applied as per type of variables. Chi square test was used for normality of data. $p < 0.05$ was considered as significant.

RESULTS

The association of majority of common clinical presentation with dengue serotypes was found as non-significant except bleeding which was found significant (Table 1). Majority of the patients were seen with normal WBC count (65%) while 35% cases were noted with leukocytopenia having WBC count $1-4 \times 10^9/L$. Thrombocytopenia was seen in the 47% cases with platelets count between $51-100 \times 10^9/L$, followed by 23% with severe thrombocytopenia between $1-50 \times 10^9/L$, 21% were found with thrombocytopenia between $101-150 \times 10^9/L$ while only 9% cases were found with more than $> 150 \times 10^9/L$ platelets.

Table 1: Association of Clinical presentation with serotypes (N = 100).

Clinical Presentation	Dengue Serotypes			
	I (n = 79)	II (n = 19)	III (n = 02)	P-value
Fever = 91	70	19	02	0.21
Body ache = 65	50	13	02	0.09
Headache = 55	39	15	01	0.24
Anorexia = 45	28	15	02	0.12
Vomiting = 15	08	06	01	0.72
Rashes = 10	05	04	01	0.50
Bleeding = 05	00	03	02	0.01

Table 2: Association of Total leukocyte count with serotypes (N = 100).

TLC Count	Dengue Serotypes			
	I (n = 79)	II (n = 19)	III (n = 02)	P-value
$1-4 \times 10^9/L = 35$	25	09	01	0.05
$> 4 \times 10^9/L = 65$	54	10	01	2.110

The association of TLC was found significantly associated with severity of serotype. There were only two cases of serotype III and both had decreased TLC ($p < 0.05$) (Table 2). Thrombocytopenia was significantly associated with serotype (Table 3).

Table 3: Association of Platelets count with serotypes (N = 100).

Platelets Count	Dengue Serotypes			
	I (n = 79)	II (n = 19)	III (n = 02)	P-value
$1-50 \times 10^9/L = 23$	12	09	02	100.0
$51-100 \times 10^9/L = 47$	39	08	00	
$101-150 \times 10^9/L = 21$	19	02	00	
$> 150 \times 10^9/L = 09$	09	00	00	
				18.798

However, no significant difference was found between hematocrit values and serotype ($p = 0.76$) (Table 4).

Table 4: Association of Hematocrit with serotypes (N = 100).

Hematocrit	Dengue Serotypes			
	I (n = 79)	II (n = 19)	III (n = 02)	P-value
Hematocrit	48 ± 4.2 (%)	47 ± 5.4 (%)	45 ± 3.8 (%)	0.76

DISCUSSION

Over the period of last 30 years, dengue fever is trending towards as emerging pandemic and more than two third of cases of occurs in Asia, Western Pacific region, America, Middle East and Africa.^{15,16} Halsey et al reported that musculoskeletal and gastrointestinal manifestations were common in individuals infected with DENV-3 while respiratory and cutaneous manifestations were more pronounced in DENV-4 infected individuals.¹⁷ Individuals infected with DENV-2 had fever, headache, retro-orbital pain, body ache, vomiting, red eye but it can cause severe disease than others, and the individuals infected with DENV-1 had fever, headache, retro-orbital pain, vomiting, red eye, and mild hemorrhage.¹⁷

In present study, we found that patients who were infected with DENV-1 and DENV-2 had fever headache, body ache, anorexia, vomiting, and rashes, while patients infected with DENV-3 had fever, headache, body ache, anorexia, vomiting, rashes and mucocutaneous bleeding. Other reports had similar findings.^{18,19}

In present study, severity of thrombocytopenia was significantly associated with severity of serotype with p-value 0.001. About 47% of the cases were found with platelets count between $51-100 \times 10^9/L$, whereas 23%

were found with severe thrombocytopenia with platelets between $1-50 \times 10^9/L$, while 21% of patients were found with platelet count between $101-150 \times 10^9/L$ and only 9% cases were found with more than 150 thousand platelets. Khan et al reported that out of 250 patients 82 patients were seen with normal platelet count, 5 patients were found with severe thrombocytopenia ($< 25,000/\mu l$), 106 patients with moderately low platelet count ($25,000/\mu l-100,000/\mu l$) and 57 cases with border line thrombocytopenia ($100,000/\mu l-150,000/\mu l$).²⁰

Mahmood et al reported that in all 43 DHF patients had platelet count ranging between $1-100 \times 10^9/L$ which is inconsistent with present study.²¹ Yung et al reported there was significant serotype specific differences for platelet count with DENV-2 cases having the lowest platelet count with a median of $114 \times 10^9/L$ compared with DENV-1 ($128 \times 10^9/L$) and DENV3 ($141.5 \times 10^9/L$) cases ($P < 0.01$).²²

In an international study, Fried et al found that there was no significant difference in the rates of DHF by serotype, whereas DENV-2 was significantly associated with increased odds of DHF grade-1 compared to DF.²³ Present study is inconsistent with Fried et al²³ and Yung et al.²² Yung et al reported that there was no significant change in hematocrit in serotype of DENV cases as DENV-143.8 (26.5-74.5, present study is consistent with this study.²²

CONCLUSION

Bleeding was only clinical presentation associated significantly with dengue serotypes. The low platelets count and TLC were also significantly associated with severity of dengue serotype. Patients with serotype III had severe thrombocytopenia.

Author Contributions:

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