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Serotype Distribution and Demographics of Dengue Patients in a Tertiary Hospital of Lahore, Pakistan during the 2011 Epidemic

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Abstract

The dengue outbreak in Lahore, Pakistan during 2011 was unprecedented in terms of its severity and magnitude. This research aims to determine the serotype distribution of the dengue virus during this outbreak and classify the patients demographically. 5ml of venous blood was drawn aseptically from 166 patients with dengue like signs to test for the virus between the months of August to November 2011. The samples were sent to CDC, Atlanta, Georgia for the purpose of molecular assays to determine their serotype. RT-PCR protocol was performed targeting the 4 dengue serotypes. Out of 166 cases, dengue infection was detected with RT-PCR in 95 cases, all infected with the same serotype DEN-2. 75 % of positive cases were males while 25 % were females. Most positive patients were in the age range of 16 - 30 years. 33 % positive cases had accompanying bleeding. This is first study during the 2011 dengue epidemic in Lahore that reports DEN-2 as the only prevalent serotype. It also indicates that more infected patients were males, adults, within the age range of 16 - 30 years, the epidemic peaked in the month of November, Dengue hemorrhagic fever (DHF) is manifested more in females, and that Ravi town was heavily hit by the dengue virus infection.

Keywords: Dengue, serotypes, Pakistan, DEN 2, Lahore, demography, serotype distribution, 2011 epidemic

Introduction

Dengue, known also as the "Break-bone" fever, is caused by a single stranded RNA virus belonging to the family Flavivaridae, of genus *flavivirus*. The virus has four subtypes on the basis of differences in serology- DEN-1, DEN-2, DEN-3 and DEN-4. It is transmitted to the human host by the bite of the *Aedes aegypti* or less commonly the *Aedes albopictus* mosquito [1]. The virus can cause three important clinical manifestations: Dengue Fever (DF), Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) [2]. It is proposed that previous infection with a different serotype may pose a risk for the development of the severe complication called DHF [3]. An estimated 50 - 100 million cases of DF and about 250,000 - 500,000 of DHF occur every year globally [4]. The average number of cases of the Dengue virus has increased from about 10,000 cases in 1950 to about 200,000 cases in South East Asia in 1990s. The morbidity and mortality from the dengue virus has generally increased over the years [5].

The major epidemic in Asia started after World War II which has created ideal environment for the transmission of the disease. Since then major outbreaks are documented in South Asia and South East Asia [6]. It has been observed that variations exist in the distribution of the serotypes

during different times. In Thailand DEN-1 was the commonest serotype in (1990 - 1992), DEN-2 (1973 - 1986, 1988 - 1999), DEN-3 (1987, 1995 - 1996) and DEN-4 (1993 - 1994). DEN-3 was the most notorious in causing serious outbreaks [7]. The records of the Dengue epidemic of 1988 in Sri Lanka showed DEN-2 as most common serotype followed by DEN-3 [8]. The major etiological agent found in the 1996 DHF epidemic in India in and near Delhi was DEN-2 [9].

The first "Documented report" of Dengue in Pakistan was made in 1985 [10]. The first "Reported outbreak" of the virus in Pakistan was recorded in 1994, when 5 cases of undifferentiated fever were diagnosed to be DEN-2 positive by Agha Khan University Hospital in Karachi [11]. Another epidemic was witnessed in Karachi following heavy rainfall in 2006 [12], affecting about 4,750 people and claiming 50 lives over a period of about six months. An epidemic of DF hit Lahore in 2008 [13], followed by an outbreak in 2010 and the most critical outbreak in 2011. Similarly, in the outbreak of Dengue in Karachi in 2005, sequences proved that the serotype involved was DEN-3 [14]. In the outbreak of Dengue virus in Punjab during 2008 a study conducted in two tertiary care hospitals of Lahore showed that DEN-4 was found in more cases followed by DEN-2 and DEN-3 [15]. The 2010 epidemic in Lahore, Pakistan showed DEN-2 was the most prevalent serotype [16]. Studying the 2008 Dengue outbreak in Lahore, researchers noted that Dengue was more prevalent in densely populated areas, in the age-group of 20 - 29 years, in males more than in females and the maximum reactive cases tended to occur in November. Other researchers also noted that males had a higher disease prevalence, and more adults were affected compared to children and that the fever peaked in November [13].

This present study explores for the first time to our knowledge, the most common serotype seen in major dengue epidemic of 2011 in Lahore. There's been no significant study on the serotype determination using molecular based techniques in the major epidemic of Lahore in 2011. We have collected the samples from patients during the active phase of the disease and the serotypes were determined. In this study, we have recorded and analysed the distribution of Dengue with respect to age, gender and location during the Lahore outbreak of 2011, by studying patients from Mayo Hospital, a tertiary hospital of Lahore.

Materials and methods

3 - 5 ml of venous blood was collected from 166 patients within the first 7 days of infection, with clinical presentation of DF (high grade fever, rash, retro-orbital pain and bleeding) during August-November 2011 (active phase of outbreak), at laboratories of the Pathology Department of King Edward Medical University (KEMU), Lahore, in collaboration with the Emergency and Accidents Department of Mayo Hospital, Lahore. The samples were collected with a proper protocol and consents were taken from the patients or their guardians. All other ethical issues were considered during the process of sampling. Moreover, an approval was taken from the Ethical Consideration board of KEMU. Samples were allowed to clot for half an hour, then samples were centrifuged and serum was separated from all the samples to be sent for investigation.

Hess or Tourniquet tests were conducted in these cases in the standard method [17]. Dengue Hemorrhagic Fever (DHF) is diagnosed on the basis of demonstrating an increased capillary permeability and thrombocytopenia with concurrent haemoconcentration. The Hess or Tourniquet test has been recommended as the initial screening procedure of patients with suspected DHF. Hematological (Complete blood count, CBC) and biochemical parameters (Liver Function Tests, LFT's and Renal Function Tests, RFT's) were also evaluated.

Data on sex, age and the presence or absence of bleeding was taken. The inclusion criteria for the study was patients of both sexes and all age groups, with high grade fever (more than 40 °C, having low platelet count (less than 100,000 cells per mm^3), patients without prior treatment, who were quite healthy before the dengue infection during that outbreak.

The samples were sent to the Center of Disease Control (CDC), Atlanta, Georgia, for the purpose of Real time PCR based molecular assays for the determination of the serotypes. All measures were taken for the proper preservation of the samples during their transport to the laboratories of the CDC. The results were then received from the CDC and the statistics were applied to the data, using SPSS version 20.

Results and discussion

The Center of Disease Control (CDC) reported back the results of all the serum samples (166) sent to their laboratories to detect the presence or absence of RNA of dengue virus. Out of 166 samples obtained from the reported active cases during that outbreak, there were 125 male and 41 females (**Table 1**) making the male to female ratio 3:1; 95/166 (57 %) samples were acute viremic for dengue infection (**Table 1**) among which 71/125 (56.8 %) were males and 24/41 (58.5 %) were females. CDC reported that all the 95 positive viremic cases had contracted the DEN-2 serotype.

Table 1 Genderwise distribution of suspected dengue patients.

Gender			Total patients
Male patients	Positive	71	125
	Negative	54	
Female patients	Positive	24	41
	Negative	17	
Total patients			166

Mean age +/- S.D. was 31.54 +/- 14 in 89/95 (94 %) of the positive viremic cases. 32/95 (33 %) positive viremic cases had accompanying bleeding. 20/71 (27 %) dengue infected males showed bleeding while 12/24 (50 %) dengue infected females displayed haemorrhagic signs. Most haemorrhagic signs were seen in the age group of 16 - 30 years. 5/95 (5 %) of the dengue infected patients fell in the age group of 1 - 15 years, 52/95 (53 %) fell in 16 - 30 years, 21/95 (22 %) came under 31 - 45 years, 15/95 (16 %) in 46 - 60 years, 2/95 (2 %) in 61 - 75 years and none > 75 years (**Tables 2** and **3**; **Figures 1** and **2**).

Table 2 Distribution of DEN-2 positive patients according to bleeding status.

Bleeding	Dengue positive cases		
	Males	**Females**	**Total**
Yes	20	12	32
No	51	12	63
Total patients	71	24	95

Table 3 Agewise distribution of DEN-2 positive dengue patients with bleeding status.

Age range (years)	Dengue positive cases				Total patients
	Males		**Females**		
	Yes	**No**	**Yes**	**No**	
1 - 15	0	3	1	1	5
16 - 30	13	24	8	7	52
31 - 45	4	13	2	2	21
46 - 60	2	9	2	2	15
61 - 75	0	2	0	0	2
> 75	0	0	0	0	0
Total patients	19	51	12	12	95

Liver and renal function tests were also performed on 74 acute viremic DEN-2 positive serums as reported in our previous study [18]. The tests were analysed for platelet count, serum alkaline phosphatase (ALP), alanine aminotransferase (ALT), urea, creatinine and albumin, protein concentrations. Most of these parameters were normal in dengue infected patients except for albumin, urea and creatinine. Hypoalbuminemia was observed in (54.05 %) of patients, low creatinine (95.94 %) and raised urea (41.89 %) values were observed in patients with dengue virus infection. No association was observed between these serum biomarkers and bleeding. From the study, impaired hepatorenal function, found to be common in dengue infected patients, was associated with declining levels of serum albumin, creatinine and raised levels of urea (**Tables 4** and **5**).

The patients from Lahore belonged to various towns and regions of the city. Out of a total 154 patients from Lahore, 44/154 (29 %) were from Ravi Town, 35/154 (23 %) from Data Ganj Baksh Town, 19/154 (12 %) from Gulberg, 17/154 (11 %) from Shalimar, 10/154 (6 %) from Samnabad, 5/154 (3 %) from Thokar Niaz Baig, 4/154 (3 %) from Lahore Cantonment, 1/154 (0.6 %) from Nishtar Town, 1/154 (0.6 %) from Waga Town and 1/154 (0.6 %) from Iqbal Town (**Table 6**; **Figures 3** and **4**).

Table 4 Normal ranges of serum biomarkers (LFT's and RFT's).

Range	ALT	Urea	Creatinine	Albumin	Protein	ALP
Normal	10 - 45 U/l	15 - 45 mg/dl	< 1.4 mg/dl	3.5 - 5.3 mg/dl	6.3 - 8.3 g/dl	110 - 130 U/l

Table 5 Liver and renal function tests in DEN-2 positive patients (highlighted columns show deranged levels).

Gender	ALT			Total	Urea			Total	Creatinine			Total	Protein			Total	Albumin			Total	AlP			Total
	H	N	L		H	N	L		H	N	L		H	N	L		H	N	L		H	N	L	
Males	9	43	0	52	19	31	2	52	0	2	50	52	12	26	14	52	2	23	27	52	5	44	3	52
Females	7	15	0	22	12	9	1	22	0	1	21	22	5	8	9	22	0	9	13	22	1	21	0	22
Total	16	58	0	74	31	40	3	74	0	3	71	74	17	34	23	74	2	32	40	74	6	65	3	74

H, N and L indicate High, Normal and Low respectively

Table 6 Town/Area distribution of suspected dengue patients in Lahore city.

Name of town/area in Lahore city	No. of patients
Ravi	44
Data Ganj Baksh	35
Gulberg	19
Shalimar	17
Samnabad	10
Tohkar Niaz Baig	5
Lahore cantonment	4
Nishtar town	1
Waga town	1
Iqbal town	1
Unknown	17
Total	**154**

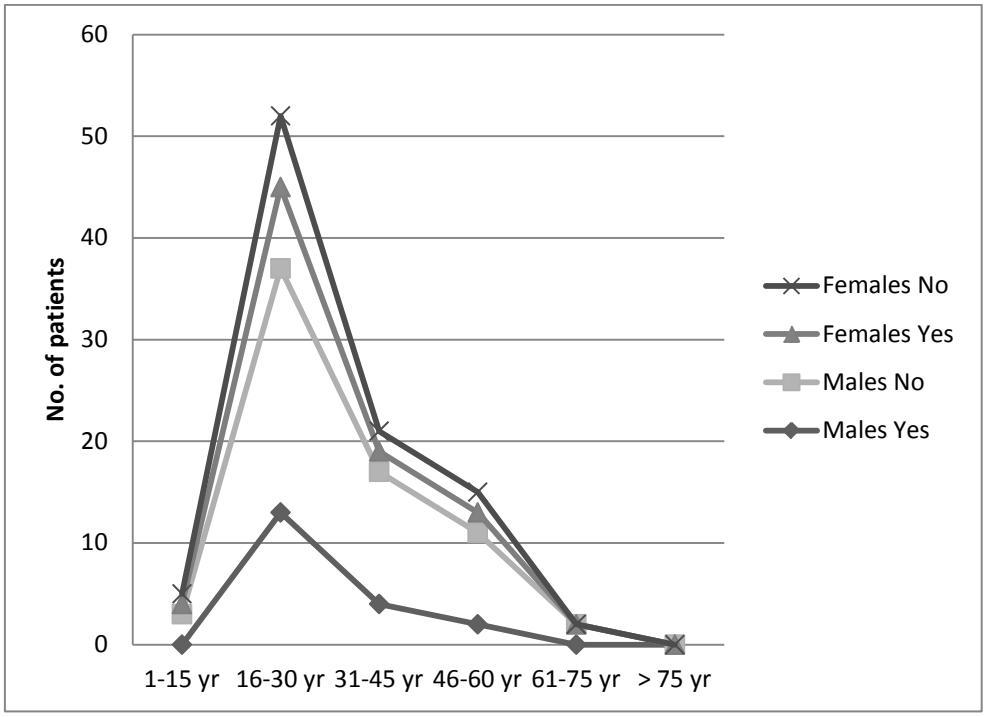

Figure 1 Age and gender wise distribution of dengue patients according to bleeding tendency. Yes and No indicates presence and absence of bleeding.

This cross-sectional analytical survey focuses on the 2011 Dengue outbreak in Pakistan that was unparalleled in terms of size as well as severity. Roughly 18,000 Dengue patients emerged nationwide with 16,000 cases and 350 deaths from the province Punjab alone [19]. Studies suggest that the emergence of Dengue virus is known to peak in the months following the monsoon season. The ideal prevalent temperature and humid conditions that follow a rainy spell, amplify vector breeding. This is concurrent with the 2011 outbreak that peaked in the post-monsoon months of September to November [13].

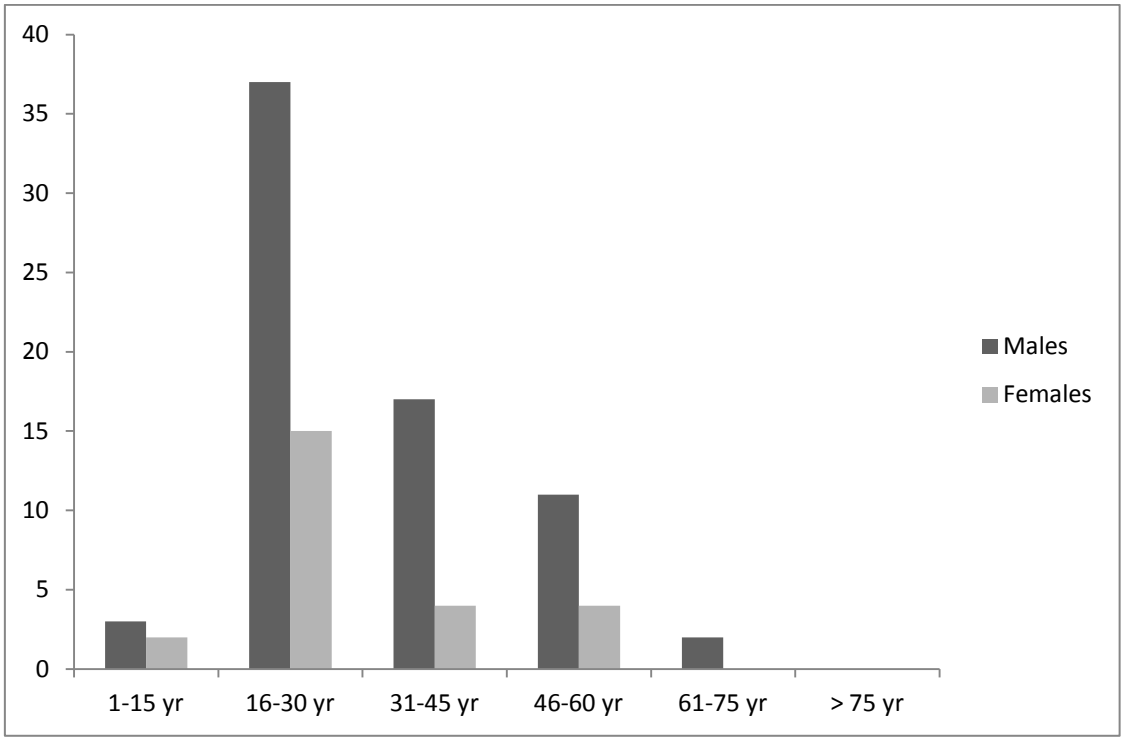

Figure 2 Age and Gender wise distribution of DEN-2 positive dengue patients.

Our results were contrary to the outbreak in the 2008 where the leading presenting serotype of the dengue virus was DEN-4, and second leading one was the DEN-2 [15]. However, the pattern changed the next year, and during the outbreaks of 2010, the most common serotype was found to be DEN-2 [16]. The pattern became exclusive during the next outbreak of 2011, which is by far the latest outbreak in Lahore, and it was found that each and every patient, whose sample was sent to CDC for serotyping, had an infection by the strain DEN-2.

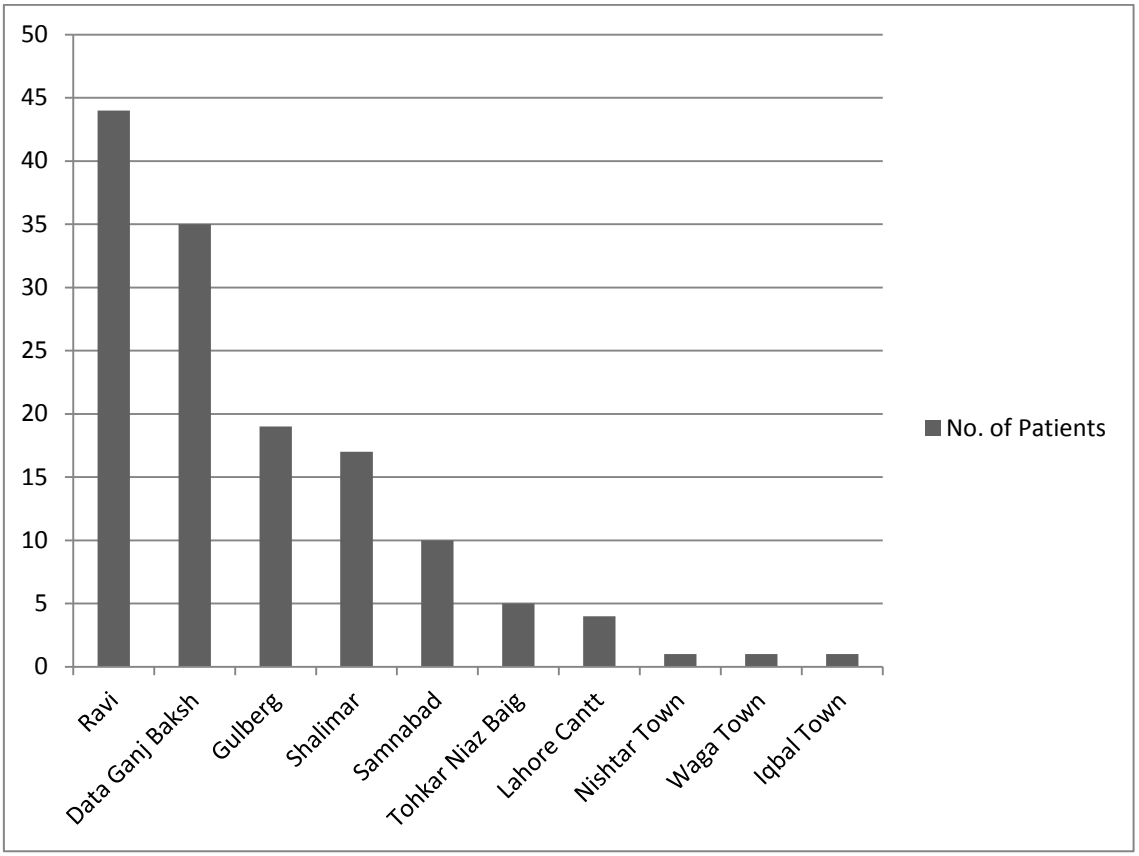

Figure 3 Dengue patients per Town/Area in Lahore.

Out of all the suspected samples submitted to the Center of Disease Control, more than half (57.2 %) were acute viremic and positive for the DEN-2 serotype. Notably, only simple and manageable inclusion criteria like high grade fever, low platelet count and the patients without prior treatment were just enough to pin-point many of the suspected patients out of which more than a half were acute viremic.

In our series, most of the Dengue inflicted patients were males. This conforms with previous results [13] and may be explained by the greater tendency of men to be bitten by the vector owing to more raw environment exposure. Another reason speculated is that females may genetically have a greater immunoglobulin producing tendency that provides them with better resistance to first disease infection. The most common age group involved was that of young adults ranging from the age of 16 - 30 years. This conforms to many previous studies and is a characteristic feature of Classic DF which is known to target older children and adults [21]. Noteworthy is that in the 2003 - 2004 Dengue infection of Karachi no child came out as sero positive for the virus [10]. Yet according to our study, (5/95) 5 % of positive cases pertained to children and teens. So over the years, the virus has paved its way into the peads and teen age group. Thus physicians should keep this phenomenon in mind, and take Dengue into account as a peads/teen fever differential. Logically, this also means that secondary infection to these children will occur at a lesser age than adults. Studies prove that the young aged incidence of secondary DF or DHF has a greater death rate [22]. Thus strong measures must be taken to especially prevent Dengue infection in children.

DHF was manifested most in individuals aged between 16 and 30, which can be accounted

for by the fact that primary Dengue infection is most likely to have occurred in this age group. DHF was found more in females than males (Odds Ratio = 2.7), in dissimilarity to previous studies [23], which may again be justified by their tendency to produce more immunoglobulins, which amplifies the antibody-dependent enhancement of the DHF variant virus [24]. Most of the patients from Mayo, a tertiary hospital, belonged to Lahore city. This was a natural happening with Lahore proving to be the most hard hit city and epicentre of the 2011 outbreak. Out of the 16,000 cases occurring in Punjab, a mammoth 14,000 were citizens of Lahore [24].

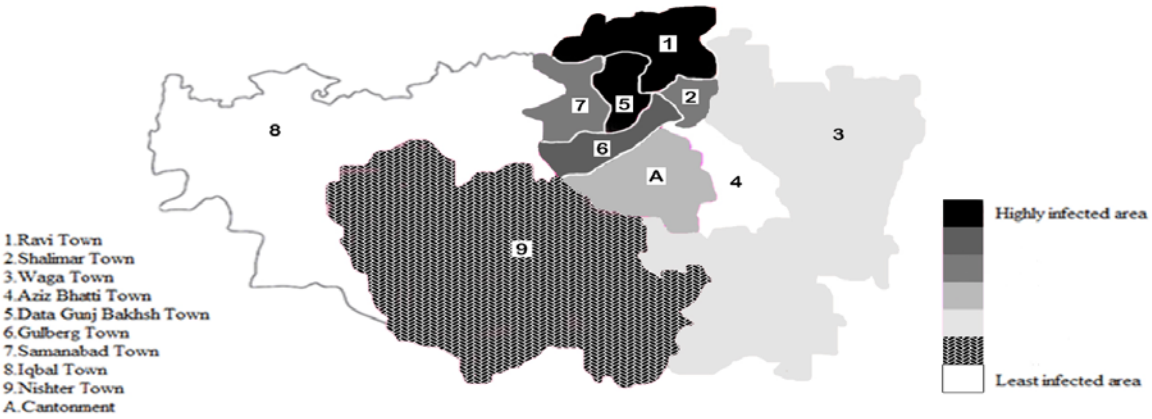

Figure 4 Townwise distribution of acute viremic dengue positive patients in Lahore (Intensity of colour shows severity of dengue virus infection).

Lahore is a cosmopolitan city, home to many immigrants and emigrants, with an estimated population of 9 million, the most in Punjab [25]. Being home to such a large population naturally, the city has numerous man made items the likes of which include tyres, domestic underground water tanks, drums and earthen pots. With Aedes showing 100 % breeding in manmade habitats, all these features make it ideal for the vast breeding of the Dengue vector. That being said, the reason for such an unprecedented outbreak of Dengue, in terms of sudden onset and magnitude, in Lahore still remains uncertain.

Cases reported from the towns and areas of Lahore varied, a look into which may provide an insight into the regions more prone to Dengue in Lahore, and the factors leading to this. The towns presenting most cases were Ravi and Data Gunj Baksh (DGB) Town. Following them were the towns Gulberg, Shalimar and Samnabad respectively. Our results conform to those concluded by the Punjab Government that designated the very same towns as the most affected by Dengue in 2011 [25]. The nearness of the Ravi River to Ravi Town may be a contributing factor to the high Dengue prevalence there. Certain areas of Samanabad Town also lie in proximity to the Ravi River. Post monsoon, a rise in the water levels of the river results in the formation of water pools in nearby sites. Such collections of stagnant flood water can be fertile sources of *Aedes aegypti* [13]. Data Gunj Baksh Town consists of a dense population distributed on inadequate space. The congestion and over crowdedness means a large number of hosts available near to each other, which suits the *Aedes* mosquito's feeding style. The town is also home to immense non-biodegradable plastic vessels, old tyres as well as water storage containers that inhabitants use due to the lack of constant water supply [13]. A survey research of Lahore in 2009 discovered that the most *Aedes* larvae were found in the used tyre markets of DGB Town [26]. All these factors add to the prevalence of Dengue in the stated town. Similarly, a large number of inhabitants, the presence of open sewage, a

plantation nursery and water pools in open land plots characterize Gulberg Town making it prone to the spread of Dengue. In contrast to the records of the Punjab Government [25], Iqbal Town did not present many cases to Mayo, but this may be justified by the fact that Jinnah Hospital is a nearer government hospital to the inhabitants than Mayo Hospital.

Reasons for this high prevalence in these areas can be due to poor sanitation, overcrowdedness, low socioeconomic status of patients, and standing water. Efforts for increased awareness, disease prevention and improved sanitation need to be executed to reduce the spread of this disease. The high incidence of infection by DEN-2 serotype in 2011 outbreak should not be neglected, steps should to be taken to control the disease and prevent a future outbreak. Moreover, introduction of new serotypes in the area must be tackled. A limitation of this survey is the degree to which the patients from Mayo Hospital represented the situation of Dengue in Lahore city, and the Punjab province as a whole owing to the selection bias.

Conclusions

It is striking that all the patients of this tertiary care hospital in Lahore during 2011 epidemic were infected with the DEN-2 serotype. It was also discovered that the virus was prevalent more in males than females, and adults as compared to children. 16 - 30 year individuals were most commonly infected and DHF was manifested more in females. A negative Hess test may not be sufficient to exclude a diagnosis of DHF in a febrile patient. Derranged liver and renal function tests were observed in dengue patients. Ravi, Data Gunj Baksh and Gulberg towns of Lahore had high disease prevalence which peaked during the month of November. Efforts for increased awareness, disease prevention and control, improved indoor and outdoor sanitation need to be executed to thwart such an outbreak from occurring again.

Conflict of interests

The authors report no conflict of interests.

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