ORIGINAL ARTICLE

Sero-surveillance of dengue in the city Lahore, Pakistan

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Abstract

Objective: To conduct sero-surveillance of dengue cases, and to find out the demographic and socio-economic status of dengue patients.

Methods: This community-based, cross-sectional, descriptive study was conducted at the Institute of Public Health, Lahore, Pakistan, from July to December each year in 2011, 2012 and 2013, and comprised patients suspected to have dengue. Blood samples of dengue patients in five randomly selected union councils of the 10 towns of the city were subjected to the dengue test. Information about demographic and socio-economic characteristics was obtained with the help of pre-tested semi-structured questionnaire. SPSS 16 was used for data analysis.

Results: Of the 5,544 dengue patients, 5.263 (94.9%) patients were diagnosed in 2011, followed by 25(0.45%) and 256(4.6%) in 2012 and 2013, respectively. Sero-surveillance of these dengue patients in 2011 showed that on an average 3,094 (58.79%) cases had primary infection, 1,506 (28.61%) had old infection, while only 663 (12.6%) had secondary infection. Similar pattern was observed in 2012 and 2013. It was found that 2,379 (42.9%) participants were aged between 15-30 years and 2,203 (39.7%) between 31-45 years. Besides, 3,618 (65.3%) participants were males and 1,926 (34.7%) females. Also, 2,865 (51.4%) participants were labourers, 2,746 (49.5%) and 2,260 (40.7%) were educated up to primary and secondary levels, respectively. Although sporadic dengue cases were recorded from July to December, most of the cases were observed in late rainy and early post-rainy seasons.

Conclusion: The incidence of dengue was highest in people of 15-45years of age, particularly males, labourers and those with low income.

Keywords: Aedes aegypti, Epidemiological surveillance, Sero-surveillance, NS1 Antibodies, ELISA. (JPMA 67: 1173; 2017)

Introduction

The incidence of dengue is increasing worldwide and dengue has become endemic in more than 100 countries in South East Asia, South Asia, Africa and South America.[1] Dengue virus, which has now spread to Asia from South Asian countries, is vectored by Aedes aegypti and Aedes albopictus mosquito.[2]

In recent years, the incidence of dengue has increased at an alarming rate in Pakistan and the disease has now become an important public health problem in the country. Like other developing countries, main reasons for dengue spread in Pakistan include rapid unplanned urbanisation, poor civic facilities, such as inadequate water supply, improper disposal of wastes, and social behaviour of the people. Additionally, rising international and domestic trade along with increased travelling, relatively limited resources and/or lack of preparedness to contain dengue epidemic have contributed to the increase in domestic and cross-border dengue transmission.[3]

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The dengue vector, Ae. aegypti mosquito, is a daytime biter, so the incidence of dengue is usually more in daytime active people who work in factories or are students, with a majority of these people falling in the age range of 16 to 50 years.[4,5] Relationship of dengue with sex has not been established; however, in South Asian countries, as the males are more exposed during daytime to dengue mosquitoes due to their nature of job in factories and open places, the incidence of dengue is higher in them in comparison to females.[4]

Seasonal incidence of dengue has been established in different studies. Although sporadic cases of dengue have been reported from July to December, its incidence is maximum in monsoon and post-monsoon season.[6]

The detection of viral antigen and consequently seroconversion of antibodies — immunoglobulin M (IgM) and immunoglobulin G (IgG) — in the serum of patient provides an important clue of dengue infection and is considered a standard serological test for the confirmation of dengue infection.[7] It is considered a simple and less expensive method as detection of dengue-specific IgM antibodies even from a single sample of serum provides a provisional diagnosis of dengue.[8] Dengue-specific IgM antibodies is likely to appear 3-5 days after infection and remained in the blood

for 1-2 months while IgG antibodies usually appear on the 7th day, become maximum in 2-3 weeks and then persists in the blood throughout life.[8]

The first serologically confirmed outbreak of dengue fever in Pakistan was reported in 1994.[3] Thereafter, various studies have reported dengue epidemics from different parts of the country.[9,10] During the last 4-5 years, situation of dengue viral transmission has been worsening in Pakistan, especially in the post-monsoon rainy period, with densely populated cities like Karachi and Lahore being under severe threat of dengue epidemics.[9,10] The number of dengue-confirmed patients in Pakistan increased from 4,500 in 2005 to more than 21,204 in 2011. The province of Punjab alone registered 21,597 confirmed cases and 365 deaths from this disease in 2011.[11]

Against this backdrop, this study was planned at the start of dengue epidemic to know the epidemiological aspect of dengue, its seasonal prevalence along with sero-surveillance by detecting dengue-specific IgM and IgG antibodies in the sera of patients suspected to have dengue.

The current study was planned to conduct longitudinal sero-surveillance of dengue by detecting dengue-specific IgG and IgM antibodies in the sera of suspected dengue cases to know the prevalence of primary, secondary and old dengue cases in different seasons. It also attempted to find out the demographic and the socio-economic status of dengue patients.

Materials and Methods

This community-based, cross-sectional, descriptive study was conducted at the Institute of Public Health, Lahore, Pakistan, from July to December each year in 2011, 2012 and 2013, and comprised patients suspected to have dengue.

All patients age, gender, occupation, education level and income level suffering from fever and two other symptoms (like aches and pains, headache, retro-orbital pain, rash, bone pain, bleeding from mouth, nose, etc.) suggestive of dengue reported in and around 200-meter radius of houses (where mosquito ovitraps were placed) in five union councils (UCs) of the 10 towns of Lahore were included in the study.

People who were not permanent residents of the study area or those who had co-morbidities (i.e. suffering from hypertension, diabetes mellitus, chronic diseases, etc) were excluded. Also excluded were relatively serious cases.

The sample size was calculated using the following formula[11]

Sample size (n) =Z2 p (100 - p) N

d2 (N-1) +Z2 p(100-p)

Where,

Z2 = value of Z at 99% level of confidence, i.e. = 6.6564

P= the prevalence of dengue disease in Pakistan, that is 28.75%[13]

d2=the margin of error = 5 % = 5 x 5 = 25

N = number of households (HH) in the area

Samples were collected from all HH calculated with the help of above formula but only confirmed dengue cases were subjected to further investigation.

The city is divided into 10 administrative zones (9 towns and one cantonment board [CB]). Each town was further subdivided into UCs. There are 146 UCs in Lahore and the number of UCs in each town varies from 12-18. From each of the nine towns, 5 UCs and five sites in CB were selected randomly for survey.

Complete blood examination of those patients who met the World Health Organisation (WHO) definition of dengue cases (like fever since last 2 days and at least two other symptoms such as aches and pains, headache, retro-orbital pain, rash, bone pain, bleeding from mouth, nose, etc.) were taken.[14] Patients whose blood examination showed decrease in white blood cells and platelets were subjected to dengue test (non-structural protein 1[NS1] antigen/antibodies enzyme-linked immunosorbent assay [ELISA]). This practice was exercised from July to December, and no data was collected from January to June due to non-availability of dengue cases during these months of the year.

The collected blood samples were sent to the dengue laboratory of the Institute of Public Health for the detection of both dengue-specific IgG and IgM antibodies. The results were obtained on the same day of blood test.

In every case, the blood of a patient was collected at least 3-5 days after the onset of fever, allowing IgM antibodies (Ab) to appear, if present.

The patients were later interviewed to obtain their demographic and socio-economic information (age, gender, occupation, educational status and income level) with the help of a questionnaire. The semi-structured and close-ended questionnaire comprised two sections: the demographic and socio-economic data. The demographic data included age, sex and occupation whereas the socio-economic data was based on educational status and HH monthly income.

Interpretation of the test was done in line with literature.[6]

If dengue-specific IgM and IgG antibodies were not present in the sera of any patients, it was considered that they were not suffering from dengue disease.

Patients in whom serum sample contained IgG antibodies were labelled an old dengue patients. These patients were affected previously and have previous antibodies (IgG) in their blood.

If IgM antibodies were detected in the serum sample, the patients were labelled primary case as they were affected for the first time and did not suffer from dengue infection previously.

Patients whose sample contained both IgM and IgG antibodies were labelled secondary dengue cases. These patients had suffered previously and therefore had IgG antibodies, but they additionally got recent infection due to which IgM antibodies appeared in their sample.

SPSS 16 was used for data analysis. A confidence level of 99% was used to estimate the outcome variables. Analysis of variance (ANOVA) was used to find association between independent and dependent variables and testing for significance. P 0.05 was considered significant.

Ethical approval for the study was obtained from the research committee of Sustainable Development Study Centre (SDSC) of Government College University (GCU), Lahore. Verbal consent from all the participants was obtained. If the age of the respondent was less than 15 years, informed consent was obtained from their parents/guardians. Confidentiality of the data obtained from the participants was maintained and the information was not shared with anybody except for academic purposes.

Results

Of the 5,544 patients, 2,379 (42.9%) were aged 15-30 years and 2,203 (39.7%) were aged 31-45 years. Only 371(6.7%)

Table-1: Demographic and socio-economic characteristics of dengue patients in all Towns of Lahore from 2011-2013.

	Towns										
	Aziz Bhatti	**Data Ghang Bakish**	**Ravi**	**Shalamar**	**Gulberg**	**Samanabad**	**Iqbal**	**Nishter**	**Wagha**	**CB**	**Total**
Age (Years)											
≤15	33	29	44	33	32	51	39	38	26	46	371
15-30	139	247	263	221	207	229	247	292	237	297	2379
31-45	275	250	191	265	219	199	193	236	170	205	2203
>45	163	71	42	45	42	35	28	50	79	36	591
Total	610	597	540	564	500	514	507	616	512	584	5544
Sex											
Male	399	407	344	369	310	334	340	406	323	386	3618
Female	211	191	196	195	190	180	167	208	189	199	1926
Total	610	598	540	564	500	514	507	614	512	585	5544
Occupation											
Housewife	50	94	60	40	90	44	52	37	34	35	536
Student	80	80	72	33	26	40	36	23	25	34	449
Labourer	263	336	306	329	261	255	266	295	257	297	2865
Professional	181	70	72	134	87	147	151	241	185	200	1468
None	36	16	30	28	26	24	18	20	11	17	226
Total	610	596	540	564	490	510	523	616	512	583	5544
Educational status											
Illiterate	51	35	26	27	24	17	32	30	26	29	297
Primary	237	239	271	267	247	269	238	353	281	344	2746
Secondary	283	297	211	241	213	204	193	224	191	203	2260
Graduate	39	27	32	29	16	24	43	9	14	8	241
Total	610	598	540	564	500	514	506	616	512	584	5544
Monthly income (Rs)											
<15000	399	228	379	288	329	296	254	495	347	384	3399
15000-<30000	174	312	129	228	122	197	202	108	149	184	1805
30000-<45000	22	47	25	36	32	15	42	17	10	11	257
>45000	15	11	7	12	7	6	8	7	5	5	83
Total	610	598	540	564	490	514	506	627	511	584	5544

CB: Cantonment board.

Table-2: Seasonal distribution of dengue patients from 2011-2013 in all towns of the city Lahore.

	Towns										
	Aziz Bhatti	Data Ghang Bakish	Ravi	Shalamar	Gulberg	Samanabad	Iqbal	Nishter	Wagha	CB	Total
Year / Season											
2011											
Early rainy (Wk: 27-32)	5	7	3	18	14	15	1	17	14	12	106
Late rainy (Wk: 33-38)	284	213	198	368	319	337	159	359	325	362	2954
Early post rainy (Wk: 39-45)	294	320	313	152	127	138	289	189	156	170	2148
Late post rainy (Wk 46-52)	3	11	8	1	1	2	7	8	6	8	55
Total	586	551	522	539	461	492	486	573	501	552	5263
2012											
Early rainy (Wk: 27-32)	0	0	0	0	0	0	0	0	0	0	0
Late rainy (Wk: 33-38)	0	3	3	2	2	1	1	2	0	1	15
Early post rainy (Wk: 39-45)	0	1	3	0	0	1	2	1	0	2	10
Late post rainy (Wk 46-52)	0	0	0	0	0	0	0	0	0	0	0
Total	0	4	6	2	2	2	3	3	0	3	25
2013											
Early rainy (Wk: 27-32)	0	0	0	0	0	0	0	0	0	0	0
Late rainy (Wk: 33-38)	2	5	1	3	8	3	2	5	4	6	39
Early post rainy (Wk: 39-45)	5	23	4	30	28	14	13	25	5	16	163
Late post rainy (Wk 46-52)	12	15	7	3	5	2	5	4	1	0	54
Total	19	43	12	36	41	19	20	34	10	22	256

CB: Cantonment board.

patients were ≤15 years old. Moreover, 3,618 (65.3%) participants were males and 1,926 (34.7%) females. Besides, 2,865 (51.7%) participants were labourers, 1,468 (26.5%) professionals, and 536(9.7%) were housewives. Also, 2,746 (49.5%) and 2,260 (40.8%) patients were literate up to primary and secondary, respectively. Further, 3,399 (61.3%) patients were earning <Rs15,000 per month while only 83 (1.5%) patients had a monthly income >Rs45,000 (Table-1).

Of all, 5,263 (94.9%) cases were reported in 2011, 25 (0.45%) in 2012 and 256 (4.6%) in 2013. Moreover, 2,954(53.3%) and 2,148 (38.7%) patients were affected in late rainy and early post rainy seasons, respectively, in 2011. The same pattern was also observed in 2012 and 2013 (Table-2).

As far as the type of infection was concerned, the ratio of patients suffering from primary infection was almost double than old cases; of all the cases reported in 2011, on an average 3,094 (58.79%) patients had primary infection in comparison to 1,506 (28.61%) patients with old infection and 663 (12.6%) with secondary infection. Similar pattern was observed in 2012 and 2013. Month-wise distribution of patients showed that the number of patients with primary infection was highest in September and October while the lowest number was observed in July, August and December in the study period (Table-3).

Table-3: Type of dengue infection in different seasons in the city Lahore from 2011-2013.

Year	Type of Infection	Months							
		July Patients (%)	August Patients (%)	September Patients (%)	October Patients (%)	November Patients (%)	December Patients (%)	Total Patients	Average %
2011	Primary	57 (53.77)	566 (60.21)	1747 (59.48)	692 (56.35)	27 (61.36)	5 (62.5)	3094	58.94
	Old	30 (28.30)	259 (27.55)	838 (28.53)	365 (29.72)	12 (27.27)	2 (25)	1506	27.72
	Secondary	19 (17.92)	115 (12.23)	352 (11.98)	171 (13.92)	5 (11.36)	1 (12.5)	663	13.31
2011	Total	106 (2.01)	940 (17.86)	2937 (55.80)	1228 (23.33)	44 (0.83)	8 (0.15)	5263	
2012	Primary	0 (0)	2 (100)	10 (58.82)	3 (50)	0 (0)	0 (0)	15	34.80
	Old	0 (0)	0 (0)	5 (29.41)	2 (33.33)	0 (0)	0 (0)	7	10.45
	Secondary	0 (0)	0 (0)	2 (11.76)	1 (16.66)	0 (0)	0 (0)	3	4.73
	Total	0 (0)	2 (8)	17 (68)	6 (24)	0 (0)	0 (0)	25	
2013	Primary	0 (0)	8 (61.53)	14 (53.84)	84 (51.53)	20 (58.82)	11 (55)	137	46.78
	Old	0 (0)	4 (30.76)	9 (34.61)	50 (30.67)	9 (26.47)	6 (30)	78	25.41
	Secondary	0 (0)	1 (7.69)	3 (11.53)	29 (17.79)	5 (14.70)	3 (15)	41	11.11
	Total	0 (0)	13 (5.07)	26 (10.15)	163 (63.67)	34 (13.28)	20 (7.81)	256	
Grand Total		4 (0.07)	348 (6.27)	2880 (51.94)	1928 (34.77)	336 (6.06)	48 (0.86)	5544	

Discussion

This study revealed that the occurrence of the dengue disease like other infectious diseases was not symmetrical. There was an epidemic-like situation with 5,263 cases in 2011 (after a massive and widespread flooding in 2010 with formation of numerous potential mosquito breeding places) in comparison to only 25 in 2012 and 256 cases in 2013. The number of dengue patients in 2011 was considerably high because of prevailing epidemic condition, but it was found to be drastically reduced in 2012 and 2013. Another factor which contributed in the lower incidence of disease in 2012 and 2013 was good surveillance, effective anti-dengue awareness campaign and insecticidal spray in all towns in these two years.

Before 2011, there were sporadic cases of dengue in this part of the world. Although there were 4,388 cases in 2010 and 120 in 2009, the disease was not properly investigated, and monitored, and remedial measures were not taken. It was believed that the disease spread to interior of the country from the port city of Karachi due to transportation of goods or travelling of a massive number of people on religious festive occasions. This might be a valid reason as hundreds and thousands of people travel inland on these occasions; however, the important reason seems to be the transferring of goods (especially used tyres) and flooding of widespread areas in this part of the country. Due to flooding, many parts of the province were inundated with flood water for many months resulting in a build-up of new potential mosquito breeding habitats. Many eggs laid by female Ae. aegypti in these places which dried later due to drainage of water might have withstood desiccation and became viable the following year in 2011 when water became available in the rainy season.

Another very important reason for the spread of the disease in the city might be the lack of provision of civic amenities. Rapid influx of people into big cities like Lahore resulted in establishment of slums on the city's outskirts. As these areas, have not been properly planned, there is no facility of proper drinking water supply, proper sanitation and hygiene available to in-dwellers of these areas. Due to lack of these facilities and the fact that people were not very much aware about the mechanism of disease transmission and spread, the presence of a few drops of rain water in discarded wrappers/plastic shopping bags could have resulted in the laying of eggs and emergence of adults within no time in rainy season. Findings of this study are supported by studies conducted by various researchers who reported that breeding habitats associated with dengue cases were strongly related with squatter settlers, inadequate drinking water supply and lack of drainage system, discarded bottles and used tyres where rain water was collected or stored.[3,14,15]

Demographic characteristics of dengue patients in all towns of the city during the study period clearly indicated that dengue infection was far more common in 15-30 and 31-45 years' age groups, with the incidence being more in the earlier group (Table-1). Reason for this could be that most people in this age group were labourers working in factories, under constructed buildings; go-down stores and places where junk, etc. were stored. It was quite possible that these people were exposed to conditions, such as presence of piles of garbage, stagnant water puddles, inadequate sanitation and hygiene, junk or other potential breeding places for dengue mosquitoes

resulting in rapid proliferation and thereby increased possibility of mosquito bites. Moreover, it was very likely that these people had not used mosquito repellents or sprayed their premises with insecticides and thus got infected by bites of infected mosquitoes. A study conducted in Taiwan revealed a relationship of dengue infection and socio-economic conditions, while another study reported that most the patients were males who belonged to 16-50 years' age group;[4,5] and a study conducted in Saudi Arabia also reported similar results.[16] A report from Brazil advocated that the risk of dengue infection increased with advancing age.[17]

Data of this study showed that the disease was relatively less common at extremes of ages i.e. in children under 15 years and in adults over 45 years in age. This contrasts with the studies[8,18,19] that reported that children were more vulnerable to dengue infection, especially students got infected by mosquitoes in playgrounds in the evening when dengue mosquitoes were active, biting these children as well as in the classrooms as the students were not dressed properly. However, it was observed that the less fortunate children who worked in factories, under-construction buildings and go-downs, and resided in these premises were most frequent victims of this disease. The most probable reason for this was that these places were the best breeding and resting/hiding places for Aedes mosquitoes and targeted the people working or living in the vicinity.[8,18,19] Few infected people were above 45 years, probably because majority of these people were not very active or stayed in houses and thus escaped from bites of infected mosquitoes or had acquired immunity against the disease. This is in contrast to the findings of a study that stated that the incidence of dengue fever and dengue haemorrhagic fever (DHF) was higher in older age groups (> 60 years) in Taiwan, while the severe cases were most common in younger age groups (<15 years) in South East Asia.[4] A study reported that the incidence rate of dengue was more common in young adults in Indonesia[20] while one study reported that children <10 years old were most commonly affected by dengue.[21] A study revealed that a majority (44.2%) of the dengue patients were between 20-30 years old[22] while another study reported that the peak age for dengue infection was 15-30 years.[23]

The next category of people infected the most was the housewives. It was observed that most these were illiterate or educated up to primary level. Most of them were ignorant about the reasons for spread of dengue infection. These women believed that mosquitoes could not spread the infection to such a great extent and it was the will of the Almighty Allah who was punishing them/us due to their/our sins and wrongdoings.

Sex distribution of dengue incidence revealed that males were affected twice in number than females in almost all towns of the city. The reason was obvious and was understandable both from economic and social norms of the society. Males are thought to be bread earners of the household and are expected to go out and earn for the family and the females are supposed to stay back and take care of household matters and the children. Due to these reasons, males who worked outdoors became target of infected mosquitoes at their work places in large number than the females who stayed back at home. Findings of the present study is supported by a report in Taiwan stating that females have lower infection rate than males.[4] In contrast to this, one study reported that the incidence of dengue was the same in males and females.[24]

This study clearly showed that the number of dengue cases were quite high in late and early post-rainy seasons in all three years. The major reason was the presence of stagnant water which provided potential breeding places for dengue mosquitoes. Seasonal transmission of dengue infection during and after monsoon months supported the findings of other researchers.[25]

This study also categorised dengue patients into three groups, i.e. primary, old and secondary. The number of dengue patients with primary infection (IgM antibodies) was highest in all three years. The most valid reason was that dengue was a new disease in this part of the world. Most of the people in the city Lahore were not exposed and so not immune to this before. Although vector-borne diseases, especially malaria, is quite common in the city for decades, Ae.aegypti was introduced in the city and its surroundings areas first time perhaps due to import of goods (especially used tyres with stagnant water and eggs, larvae/pupae of dengue mosquitoes) or due to frequent travelling of dengue-affected patients from Karachi.

This study showed that 58.94%, 34.80% and 46.78% cases had primary infection in 2011, 2012 and 2013, respectively. In contrast to this, one study reported that 19.7% patients were suffering from primary infection.[18] Findings of this study were also in contrast to those of other studies which showed that number of old (IgG antibodies) and secondary cases (both IgM and IgG antibodies) were more than primary cases.[6] Reason of this could be that dengue was endemic in areas where these studies were performed. So, people in these areas had previous attacks of this disease with increased the number of old and secondary dengue infections.

Conclusion

The incidence of dengue was highest in 15- to 45-year-old

people, particularly males, labourers and those with low income. The increased incidence of dengue cases was observed in late rainy and early post-rainy seasons. Overall, primary cases exceeded the old and secondary cases. It is highly recommended that labourers should be advised to protect themselves from exposure during monsoon and awareness campaigns should be conducted with low-income groups as primary targets. It is important that potential breeding places in the monsoon season should be eliminated and protective measures taken so that infective mosquitoes cannot bite healthy people and transmit dengue infection.

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