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Asymptomatic dengue infection in adults of major cities of Pakistan

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ABSTRACT

Objective: To determine the asymptomatic dengue infection in adults of Pakistani population.**Methods:** This study was conducted in five major cities (Islamabad, Karachi, Lahore, Multan, and Peshawar) of Pakistan. A total of 5 230 adults aged 18 years and above without a history of dengue fever at any point in their life were enrolled from participating laboratories. Those who were confirmed for dengue previously were excluded. Of the total, 62.6% ($n = 3\ 276$) were male with an average age of 34.6 years. Participants were briefed about the objectives of the study, and written consent was obtained to perform dengue IgG test using enzyme linked immunosorbent assay. The brief information related to age, gender and area was also taken on proforma.**Results:** Overall 32.3% ($n = 1\ 691$) was having asymptomatic dengue infection which was 67.5% ($n = 756$) in Karachi followed by 39.1% ($n = 391$) in Islamabad, 29.9% ($n = 316$) in Lahore and 21% ($n = 228$) in Peshawar and none from Multan. More males were affected with asymptomatic dengue infection than females. The asymptomatic dengue infection was significantly higher in different cities; however, there was no significant difference with respect to age groups.**Conclusions:** The asymptomatic dengue infection is higher in cities *i.e.* Karachi, Islamabad and Lahore which are at risk of developing secondary dengue infections. There is a need of awareness among the public about secondary dengue infection.

1. Introduction

Dengue is a mosquito borne viral infection that causes mild to severe illness. Dengue virus is a positive stranded RNA virus which belongs to family Flavi-viridae. It is composed of four serotypes including dengue virus (DENV) 1, DENV-2, DENV-3 and DENV-4. These serotypes are genetically related but antigenically distinct [1,2]. It is transmitted to host via mosquito vector *Aedes aegypti*. Infection with dengue virus can lead to a spectrum of diseases ranging from sub-clinical infection to dengue fever and

most severe forms like dengue hemorrhagic fever and dengue shock syndrome [3]. About 50–100 million infections occur in over 100 endemic countries and it is estimated that almost half of the world's population are at risk of dengue [4]. Many countries in the world have high incidence, and dengue epidemics have spread almost 120 countries [5].

In Pakistan, the first dengue epidemic was reported in 1994 [6]. Since then, outbreaks of dengue infection have been reported in different age groups and from various regions of Pakistan [7,8]. Major dengue epidemic in Lahore in 2011, resulted in hundreds of deaths and affected thousands. The majority of deaths occurred due to co-morbidities [9] like diabetes and lack of awareness among physicians for its management [10]. It was reported that dengue was responsible for 133.76 disability-adjusted life years lost per million population [11].

The symptoms of dengue fever include high-grade fever, headache, myalgia and pain in joints [12]. The dengue

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haemorrhagic fever is mostly seen in secondary infection and has symptoms including haemorrhagic episodes (petechiae, purpura, ecchymosis) in addition to high grade-fever [13,14]. Generally, the dengue infection recover completely confers lifelong immunity against specific dengue serotype. The dengue secondary infection with different serotype generates cross reactive antibodies which increase the risk of antibody dependant enhancement of disease [15]. The recurrent dengue infection is an important risk factor for serious and fatal complications of dengue haemorrhagic fever. In secondary infection, the responses of T cell against previous dengue infecting serotype do not give protection and also are associated with immune responses that induce severe symptoms [16].

The asymptomatic dengue infection is defined as having no clinical signs or symptoms of disease *i.e.* dengue fever, dengue haemorrhagic fever [17]. The asymptomatic dengue infection gives rough estimate of individuals who need strict vigilance and personal protection. The viremia in asymptomatic cases potentially forms an important virus reservoir for transmission of infection especially in areas where vector population is high [18]. Different studies have reported prevalence of asymptomatic dengue infection among blood donors [19,20], patients [21] and population [22–24]. Similarly, from Pakistan, the prevalence of asymptomatic dengue infection was reported in healthy individuals (28.8%) [25], health care professionals (7.7%) [26] and children (25%) [27]. This study was planned to assess the asymptomatic dengue infection among adults in different cities of Pakistan.

2. Materials and methods

2.1. Ethical standards

The study was approved by Institutional Bioethics Committee of Pakistan Health Research Council, Islamabad. Written informed consent was taken from all the participants. All procedures contributing to this work comply with the Helsinki declaration.

2.2. Sample size and target population

This was a cross-sectional multicentre study which was conducted in five major cities *i.e.* Islamabad, Karachi, Lahore, Peshawar and Multan of Pakistan. A sample size of 5 230 was calculated on the basis of previously reported prevalence *i.e.* 28.8% [25] with 5% precision and 99% confidence interval. The target population was comprised of adults aged 18 years and above who did not have the history of fever or any other dengue related symptoms at any point in their life.

A total of 5 230 subjects were enrolled including 998 from Islamabad, 1 120 from Karachi, 1 056 from Lahore, 1 087 from Peshawar and 969 from Multan. Among them, 62.6% ($n = 3 276$) were male and 37.4% ($n = 1954$) were female. About 2 441 (46.6%) were of 18–30 years age group followed by 1 469 (28%) were of 31–40 years age group and only 153 (2.9%) were of more than 60 years age group.

2.3. Specimen collection and testing

The participants were enrolled from selected laboratories in each city using convenient sampling. In Karachi, Lahore,

Peshawar and Multan, adults coming to Pakistan Health Research Council Research Centre Laboratory were attached with tertiary care hospitals while in Islamabad, adults coming to CITILAB Labs were enrolled. Participants were asked about the history of fever or any other dengue related symptoms at any point in their life using a standard proforma. Those who confirmed any dengue related symptom were excluded.

The selected participants were briefed about the study procedures and their consent was taken to use the blood taken for routine lab testing for estimation of dengue IgG. Brief information related to age, gender and address was taken using a predesigned proforma. Blood samples were stored at -20°C until use. For the determination of dengue IgG, commercially available kit *i.e.* DENV IgG ELISA kit (Cortez Diagnostics USA) was used. All tests were performed in batches as per manufacturer instructions along with the positive and negative controls.

2.4. Data entry, interpretation and analysis

The data was entered and cleaned using Microsoft Excel 2007 (Microsoft Corporation). The cut-off was calculated by multiplying the mean absorbance of the calibrator with the calibrator factor. Further, the antibody index for positive and negative control was calculated from the absorbance of each control and a cut-off value of the assay batch. As per the manufacturer instructions, values <0.9 were considered negative for DENV IgG and $0.9-1.1$ were considered as borderline-positive while values >1.1 were positive for DENV IgG. Those who had positive dengue IgG were defined as having asymptomatic dengue infection [17].

The data was analyzed using SPSS version 15 where *Chi-square* test was used to determine the relationship between age groups, genders and cities. $P < 0.05$ was considered statistically significant.

2.5. Geo mapping of dengue IgG positive cases

The geo-mapping of dengue IgG positive cases was done using spatial epidemiology.net software [28]. The addresses of the participants were fed in the software which geocoded the addresses and gave latitude and longitude. Map was created by the software by using the latitude and longitude.

3. Results

3.1. Asymptomatic dengue infection of cases

Overall 1 691 (32.3%) of the subjects were having asymptomatic dengue infection. Among them, the majority 756 (67.5%) was from Karachi followed by Islamabad 391 (39.2%), Lahore 316 (29.9%) and Peshawar 228 (21%). However there was no dengue asymptomatic case from Multan. The asymptomatic dengue infection was higher in Karachi (Table 1).

Overall, asymptomatic dengue infection was more (34.5%) in females as compared to males (31%) (Table 1). When comparing among cities, asymptomatic dengue infection was more in males *i.e.* 40% males versus (*vs*) 30% females in Islamabad, 22% males *vs* 20% females in Peshawar and 70% males *vs* 65% females in Karachi. However in Lahore, females were more (31%) than males (29%).

When the asymptomatic dengue infection was compared with age groups and gender, there was no significant difference

Table 1

Demographic characteristics of participants [n (%)].

Categories	Total number	Asymptomatic dengue infection
Male	3 276 (62.6)	1 015 (31.0)
Female	1 954 (37.3)	676 (34.5)
Age groups		
18–30 years	2 441 (46.6)	674 (27.6)
31–40 years	1 469 (28.0)	451 (30.7)
41–50 years	843 (16.1)	318 (37.7)
51–60 years	324 (6.1)	181 (55.8)
>60 years	153 (2.92)	67 (43.7)
Cities		
Overall	5 230	1 691 (32.3)
Islamabad	998	391 (39.1)
Karachi	1 120	756 (67.5)
Lahore	1 056	316 (29.9)
Peshawar	1 087	228 (21.0)
Multan	969*	0

* None was positive for Dengue IgG.

Table 2

Difference of asymptomatic dengue infection in gender, age groups and cities.

Factor	Male	Female	Total
Age groups (years)			
18–30	338	228	566
31–40	256	174	430
41–50	214	153	367
51–60	140	71	211
60+	67	50	117
Cities			
Islamabad	357	34	391
Karachi	335	421	756
Lahore	202	114	316
Peshawar	121	107	228

observed between genders ($\chi^2 = 4.405, P = 0.353$). However, there was a significant difference in asymptomatic dengue infection among different cities of Pakistan ($\chi^2 = 243.81, P < 0.001$) (Table 2).

3.2. Mapping of asymptomatic dengue infection cases

The mapping of asymptomatic dengue infection has shown that the cases were widely spread all around the cities and there was no specific cluster for the disease (Figure 1).

4. Discussion

This study reported asymptomatic dengue infection residing in Pakistan with variation in different cities. More females were exposed to dengue and young adults having age ranging from 18–30 years were more affected as compared to other age groups.

Overall, there was high asymptomatic dengue infection in the country. It has been shown that in Pakistan, all four serotypes of dengue are present but DENV-2 and DENV-3 serotypes are more prevalent [29–31]. It was reported that suffering from one dengue serotype confers lifelong immunity to that serotype but at the same time make results in severe outcome if infected with another serotype [15,16]. The current study showed that high prevalence of asymptomatic dengue infection, emphasis on the point that if any other serotype like DENV-1 or DENV-4 infects, will result in serious complications of the disease and will result in the increased morbidity and mortality.

As compared to cities, asymptomatic dengue infection was higher among Karachi as compared to other cities. The dengue infection was first reported from Karachi in 1994 [32] and then later appear in 2006. The serotypes were DENV-2 and DENV-3 [29] were predominant and none was for DENV-1 and DENV-4. The recent study showed about 67% prevalence of

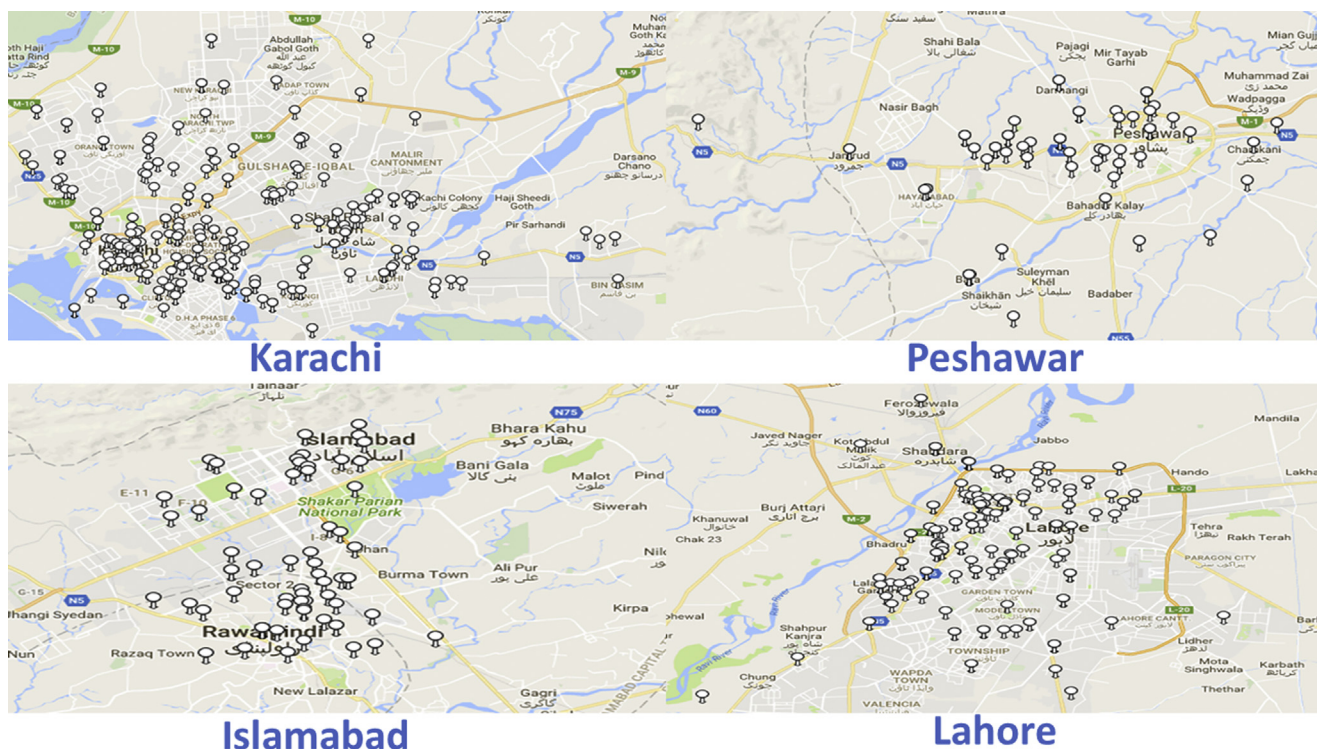


Figure 1. Asymptomatic dengue infection in four major cities of Pakistan.

asymptomatic dengue infection in Karachi which was spread all over the city, indicating a potential threat of severe dengue will come if any of the other serotypes *i.e.* DENV-1 or DENV-4 enters the city. Therefore, there is a need to take precautionary measures as more than half of population have already got exposure to the dengue virus.

Overall, more females were exposed to dengue than males, however, when comparing between cities, male preponderance was observed in Islamabad, Karachi and Peshawar while in Lahore females were more affected with asymptomatic dengue infection than males. The reasons for this might be because females cover most of their body and mostly stay indoor [33]. Studies conducted in other dengue endemic countries on different groups like blood donors, general population, *etc.* also showed that asymptomatic dengue infection was more in males [24,34–36]. Similarly, in the current study, the prevalence of asymptomatic dengue infection was common in young adults. The community based studies were done in healthy population also showed a high prevalence of asymptomatic dengue infection in young adults [22,23,26,36]. This shows that male especially young adults are at more risk of developing asymptomatic dengue infection. Therefore, there is a need to have a gender specific approach to address; especially adults may be formulated to cope with this issue.

The prevalence of asymptomatic dengue infection had been reported from countries having dengue epidemic which varied from country to country. The community based studies in Sudan and Zambia showed 27% and 4% prevalence of asymptomatic dengue infection in general population respectively [23,24]. Studies among blood donors also showed variation in prevalence of asymptomatic dengue infection *i.e.* 92% in Puerto Rico [19], 50% in Tanzania [34] and 14% in Kuwait [20]. Similarly, the prevalence of asymptomatic dengue infection among healthy adults coming to a laboratory for routine check up also was reported 89.5% in Andhra Pradesh, India [35]. These all studies show that asymptomatic dengue infection is common in dengue epidemic countries and there is a potential threat of deadly dengue epidemics due to secondary infection. There is a need to develop a strategy and guidelines to cope with this issue and avert any future deadly dengue epidemic.

The asymptomatic dengue infection is high in dengue endemic countries as shown in this study (32%) which indicates a potential threat of deadly epidemics especially in Southeast Asia where cyclic dengue epidemics have been seen [28]. There is a need to have integrated approach including awareness among the community and preventive measures in order to reduce the exposure, which will eventually help in reducing the dengue burden.

The study has some potential limitations including the recall of the participants for previous dengue history, not doing serotyping and enrollment from the labs, therefore, the findings of the study cannot be generalized.

Conflict of interest statement

The authors declare that they have no competing interests.

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