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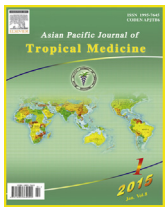

Original research https://doi.org/10.1016/j.apjtm.2017.09.013

# Asymptomatic dengue infection in adults of major cities of Pakistan

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ARTICLE INFO

*Article history:*
Received 11 Jul 2017
Received in revised form 18 Aug 2017
Accepted 7 Sep 2017
Available online xxx

*Keywords:*
Dengue
Exposure
Secondary dengue infection
Asymptomatic

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-viradae. 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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Foundation project: The study was funded by Pakistan Health Research Council.



Please cite this article in press as: Rafique I, et al., Asymptomatic dengue infection in adults of major cities of Pakistan, Asian Pacific Journal of Tropical Medicine (2017), https://doi.org/10.1016/j.apjtm.2017.09.013

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

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