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## Short Communication

# Change in demographic pattern of dengue virus infection: evidence from 2011 dengue outbreak in Punjab, Pakistan

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shift in dengue viral infection in countries in South East Asia, implying that the epidemiology of this disease is changing.<sup>3</sup>

Different studies have reported contradictory information on the incidence of dengue viral infection in males and females. Some studies have reported equal prevalence rates in males and females, while others have reported higher prevalence rates in females or males.<sup>4–6</sup> A major outbreak occurred from June to November 2011, where the metropolitan city of Lahore was a key affected area followed by Faisalabad and other districts. The present study was undertaken to study dengue viral infection by gender and age group in Punjab, Pakistan during the 2011 outbreak.

## Introduction

Dengue viral infection is a global challenge. Due to global distribution of its vector (*Aedes*), interepidemic phases have condensed and more serious cases of dengue illness are being reported, especially in South America and Asia where it has become endemic. Dengue epidemics have been reported occasionally in Pakistan, with involvement of all four serotypes. The first documented outbreak of dengue viral infection occurred in Karachi in 1994, followed by another epidemic in 2005. Most reported deaths in a major outbreak of dengue infection in 2006 in Karachi were due to Serotypes 2 and 3. There have been many sporadic cases of dengue viral infection in Punjab, with a major outbreak in 2003. Many cases of dengue fever have subsequently been reported in this province.<sup>1,2</sup>

Age and gender are considered to be major factors in dengue viral infection. Many studies have reported an age

## Methods

Over the course of the 2011 outbreak in Punjab, 1161 serum samples from patients that showed signs of fever, rash and muscular ache were received from local hospitals. Of these samples, 495 had complete demographic and clinical data, and were included in the study. Dengue virus serotyping was performed by polymerase chain reaction (PCR) amplification of the *C/prM* gene junction, as described previously.<sup>1</sup> Briefly, viral RNA was extracted using a GF-1 Viral Nucleic Acid Extraction Kit (Vivantis Technologies Sdn Bhd, Kuala Lumpur, Malaysia) by the extraction protocol given in the kit manual. Extracted RNA was subjected to PCR for cDNA synthesis using left primer. Between 20 and 50 ng of extracted RNA was added to 4 µl of 10X first strand buffer, 0.5 µl of 0.1 M dithiothreitol (DTT), 2 µl of

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10 mM dNTPs and 1  $\mu$ l of 20 pM left primer. Eight units of RNase inhibitor and 200 units of M-MLV reverse transcriptase enzyme (Invitrogen Biotechnologies, USA) were added. The volume was made up to 20  $\mu$ l with distilled water. The reaction mix was incubated at 37 °C for 50 min, followed by enzyme inactivation at 95 °C for 3 min. The required *C-prM* gene was amplified by nested PCR, where 50–100 ng of cDNA was added to 2  $\mu$ l of 10X PCR buffer [(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> + Tris-HCl], 2.4  $\mu$ l of 25 mM MgCl<sub>2</sub>, 1  $\mu$ l of 500  $\mu$ M dNTPs, 1  $\mu$ l of 20 pM right and left primers, 5.6  $\mu$ l of dH<sub>2</sub>O and 2 U of *Taq*-DNA polymerase enzyme (Invitrogen Biotechnologies, California, USA). The first PCR run using outer right and outer left oligonucleotides was performed at 94 °C for 2 min for initial denaturation, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 52 °C for 45 s and extension at 72 °C for 2 min. The second run was performed using type-specific inner right and inner left oligonucleotides. The cycling profile was the same as described for the first round, except the annealing temperature was reduced to 54 °C. For analysis, the available data were divided into seven age groups: 1–15, 16–25, 26–35, 36–45, 46–55, 56–65 and  $\geq$ 66 years. Pearson's Chi-squared test and one-way analysis of variance were applied to compare the means between groups using Statistical Package for Social Sciences Version 16.0 (SPSS Inc., Chicago, IL, USA). A one-sample t-test was used to test the gender distribution for significance. A P-value less than 0.05 was considered to indicate significance. The median age of infected patients was evaluated. As the distribution of ages was skewed ( $0.811 \pm 0.11$ , Shapiro-Wilk  $P < 0.05$ ), median age  $\pm$  interquartile range is reported in this study.

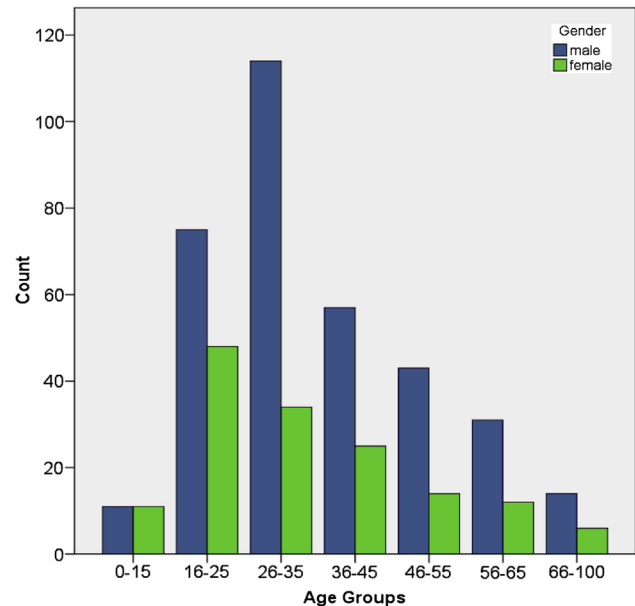
## Results and discussion

### Prevalent serotype of dengue virus

Four hundred and ninety-five samples met the study criteria and were serotyped for all four serotypes of dengue virus in separate PCR reactions. Serotype 2 was the dominant serotype, with 100% prevalence in these positive samples. Only 26 samples had concurrent infection with Serotypes 2 and 3. No infection due to Serotype 1 or 4 was detected in this dengue outbreak.

### Statistical analysis of demographic data

Of the 495 characterized cases of dengue viral infection, more males (69.7%) were infected compared with females (30.3%) ( $P < 0.01$ ). Furthermore, infected males and females were evaluated in different age groups, and males were consistently found to have higher infection rates than females ( $P = 0.039$ ). The male:female ratio (2.3:1) remained the same in all age groups  $\geq$ 16 years; however, equal numbers of males and females were infected in the  $\leq$ 15 years age group (Fig. 1). Most infected patients (59.2%) were aged between 16 and 35 years. The mean  $\pm$  standard deviation age of the infected cases was  $31.6 \pm 16.1$  years (95% confidence interval 30.1–33.0). Only 4.0% of patients were aged  $\geq$ 66 years, and only 4.4% of patients were aged  $\leq$ 15 years (Fig. 1). The median age of infected patients was  $28 \pm 20$  years, and the median age of infected females ( $25.0 \pm 22.0$  years) was far less than the median age of infected



**Fig. 1 – Number of cases of dengue viral infection ( $n = 495$ ) sorted by gender in different age groups. Only 4.4% of cases were in patients aged 0–15 years, whereas over half (59.2%) of infected patients were aged between 16 and 35 years. Equal numbers of males and females were infected in the  $\leq$ 15 years age groups. All other age groups had more male cases than female cases.**

males ( $28.0 \pm 20.0$  years). Age distribution was positively skewed to  $0.811 \pm 0.11$  from normality (Shapiro-Wilk  $P < 0.05$ ).

A study conducted on data from six countries with different economic and cultural backgrounds showed that more males in all age groups were infected than females.<sup>7</sup> Between 1996 and 2003 in Sri Lanka, 55.9% of studied cases were males. Between 1999 and 2006 in Singapore, 60.7% of infected cases were males aged  $\geq$ 15 years.<sup>7</sup> Consistent with these findings, more males were infected with dengue virus in Punjab (Fig. 1). However, the gender-dependent pattern of infection found in this study was in contrast to that found in a study in South America, where more females were infected with the virus.<sup>5</sup> In the present study, the gender distribution of infected cases was equal in patients aged  $\leq$ 15 years. A previous study from Pakistan reported a higher percentage (61%) of seropositive males than females between 2003 and 2007.<sup>8</sup> This trend progressed, with more cases of IgM-positive females being reported gradually over the study period ( $P = 0.253$ ).<sup>8</sup>

Dengue infection is considered to be a paediatric disease.<sup>9</sup> In Sri Lanka, children accounted for 60% of reported cases in 1996–1999, and this decreased to 40% in 2001–2005.<sup>7</sup> In the present study in Punjab, adults accounted for more cases of dengue viral infection, which is in agreement with other recent studies in South East Asia.<sup>10</sup> Khan and colleagues reported a decrease in the median age of dengue-infected patients from 32.0 years in 2003 to 24.0 years in 2007 ( $P = 0.001$ ), and hypothesized that acquired immunity has increased in the adult population due to earlier exposure as Serotype 2 has been circulating in the region since 2004. As Serotype 2 was first documented in Karachi in the 1994 outbreak of dengue

haemorrhagic fever, the median age of infected patients may have decreased due to circulation of this serotype in the region for many years.<sup>8</sup> However, a major outbreak due to Serotype 2 was reported in 2003 in Punjab, which may explain the higher median age of 28 years in the Punjab population.<sup>2</sup> In addition, the median age of female dengue patients was significantly lower than that of male patients (25.0 years vs 28.0 years), which is the same trend observed by Khan and colleagues (24.2 years vs 25.9 years;  $P = 0.001$ ).<sup>8</sup> They reported that the highest proportion of seropositivity was seen in patients aged 26–40 years in 2003 and 2004, and this trend shifted to patients aged 11–25 years in 2005–2007. However, in the present study, 24.8% of patients were aged 16–25 years and 29.9% of patients were aged 26–35 years (Fig. 1). In the case of dengue viral infection, in addition to several viral and host factors, everyday life settings play a significant role in exposure to *Aedes* mosquitoes. Fewer infected females and children indicates that households are safer than the outside environment.

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### Ethical approval

The study was approved by the Ethical Committee of Molecular Virology Division of the University of the Punjab. Informed consent was obtained from each participant (aged  $\geq 18$  years) or head of family (for patients aged  $< 18$  years) in accordance with the Declaration of Helsinki.

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### Competing interests

None declared.

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