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Brief Original Article

The emergence of Dengue Fever in Sheikhupura, Pakistan: its seroprevalence and risk factors assessment during 2014-2017

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Abstract

Introduction: District Sheikhupura encountered its first dengue outbreak in 2014 but lacked serological evidence and reports of risk factors associated with it. To assess this, a hospital-based study was conducted from January 2014 to December 2017.

Methodology: Blood from 333 participants was collected, the serum obtained was tested for IgG and IgM antibodies against DENV using a commercially available ELISA kit.

Results: The results showed that out of all (n= 333) samples tested, 120 were turned up positive for DENV, making an overall prevalence of 36%. Of the 120 confirmed cases, 55% (n = 66) were recorded in 2014, 10% (n = 12) in 2015, 27.5% (n = 33) in 2016, and 7.5% (n = 9) in 2017. It was found that 68.3% (n = 82) were male and 31.7% (n = 38) were female, with 61% (n = 74) patients aged between 11-30 years. The highest prevalence of infection, 94.2% (n = 113), was noted after the rainy season. During the study, the highest number of cases appeared in Ferozewala Tehsil. The factors age, gender, and season were found statistically significant with the prevalence of infection ($p < 0.05$).

Conclusions: The study is the first report on the detection of dengue in the Sheikhupura district. The survey anticipated its geographical expansion, determined associated risk factors, and suggests active disease surveillance in the area.

Key words: Dengue; prevalence; IgM; IgG; Pakistan.

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Introduction

Dengue is one of the most rapidly spreading mosquito-borne viral diseases, with around 400 million infections [1]. There are 96 million symptomatic cases, which account for 12,500 deaths worldwide each year [2]. Dengue infection is attributed to a substantial public health burden and puts two-thirds of the world's human population at risk [2]. There are several factors associated with this rise in dengue incidence. Among them, rapid population expansion, haphazard urban development, poor water storage, and inadequate sanitation contribute to its exacerbation [3]. The dengue virus (DENV) gets transmitted by the female *Aedes* mosquitoes [4], which are more often reported in urban zones but are still found in rural localities [5]. DENV infection can be asymptomatic or exhibits diverse clinical presentations, ranging from mild symptoms to life-threatening forms [4]. Currently, there is no specific treatment for DENV infection, and a preventive vaccine is yet undergoing further evaluation [6].

In Pakistan, dengue is primarily considered an urban and semi-urban disease because of the abundance of principal vector mosquitoes *Aedes aegypti* and *Aedes albopictus* in these territories [7]. From 2006 to 2011, 40,987 cases of dengue were reported, including 490 deaths [8]. In August 2013, a massive dengue outbreak broke out in Khyber Pakhtunkhwa province, affecting more than 7,000 individuals with 26 deaths [9]. During

2019, there were 47,120 confirmed dengue cases in the country, including 75 deaths. Punjab province alone contributed 21% (9,676) to the total dengue cases in the country, while 16 deaths occurred during the same period [10].

Previously, studies reported the dengue epidemic in Peshawar [11] and Multan [12] in Pakistan. Earlier, dengue got documented from Sheikhupura's neighbouring city of Lahore [13] but, no report surfaced from Sheikhupura. The present study aims to generate baseline data on the prevalence of dengue fever and its associated risk factors in febrile patients in the health facilities of Sheikhupura. The study assessed the prevalence of immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies in patients who attended health facilities of Sheikhupura and its environs. The findings of this study will facilitate healthcare professionals in the better clinical management of patients and vector biologists to devise more efficient vector control measures.

Methodology

Study area

Sheikhupura is a district in the Punjab province of Pakistan having five tehsils, namely Ferozewala, Sheikhupura, Muridke, Sharaqpur, and Safdarabad (Figure 1). According to Pakistan's latest nationwide census, Sheikhupura district has a population of 34,60,425. The climate of the study district is subject to extreme fluctuations. From mid-December to mid-March, the air is humid, cold, and the area receives intermittent light to moderate rainfall. In April, the temperatures rise sharply, and two consecutive months of extreme temperatures are experienced, followed by a monsoon until the end of June. The average annual rainfall in the district is about 635 mm [14].

Figure 1. Map of district Sheikhupura, showing possible geographical expansion pattern of DENV infection in the study area. (https://sheikhupura.punjab.gov.pk/skp_tehsils).

Study design and period

A healthcare-based cross-sectional study was conducted from January 2014 to December 2017. The participants included all patients who were showing acute febrile illness during the study course. A febrile patient was defined as a patient who came to the outpatient, the pediatric or medicine unit at the participating hospitals (DHQ hospital Sheikhupura, Children hospital, Government teaching hospital Shahdara, and Mayo hospital) with persistent high fever ≥ 38 °C.

Data collection, sample collection, and serological assays

The data were taken with the help of a pre-designed closed-ended questionnaire and documented in an Excel database. Venous blood (3mL) was collected aseptically from each participant, followed by labelling and centrifugation at 3000 rpm for 5 mins for serum separation. The obtained serum was tested for IgG and IgM antibodies against DENV using an ELISA kit (Diagnostic Automation; Cortez Diagnostics, Woodland Hills, CA, USA) following the manufacturer's protocol.

Distribution of samples

According to the intensity of rainfall, we divided the weather data into three seasons, namely pre-monsoon season (January-June), monsoon season (July-August), and post-monsoon season (September-December). The study population consisted of ≤ 10 years, 11- 20 years, 21- 30 years, 31-40 years, 41- 50 years, 51- 60 days, and ≥ 60 years of age.

Analysis of data

Graphics and statistical analyses were carried out using Microsoft Excel (2010) and SPSS 16.0. The prevalence of dengue infection was the dependent variable, while the climatic factors, gender, and age groups were independent variables. The association between DENV positivity and independent factors were tested using the χ^2 test. All differences were considered significant at p values < 0·05.

Ethical considerations

This study was conducted with the approval of the Executive District Officer of Health, Sheikhupura, on 23rd January 2019 under the decision number EDO-H/1-18/2019. Data, including names of patients, were

treated confidentially and used for research only. Before being included in the study, the patients were informed and they voluntarily agreed.

Results

During the period from January 2014 to December 2017, a total of 333 suspected cases were reported. Of them, 120 patients were tested positive, with 68.3% (n = 82) males, while 31.7% (n = 38) females. Of the all-confirmed patients, 55% (n = 66) were recorded in 2014, 10% (n = 12) in 2015, 27.5% (n = 33) in 2016, and 7.5% (n = 9) in 2017 (Figure 2). The data obtained demonstrated that of the most affected individuals, 61% (n = 74) were between the ages of 11-30 years (Table 1). The study identified that the highest number of cases, 94% (n = 113), were recorded during the post-monsoon period (Figure 3). The sera of 45% of patients (n = 54) were positive for IgM whereas, only 4.2% (n = 5) were detected positive for IgG, while 50.8% of patients (n = 61) were positive for both IgM and IgG. The gender, ages, and type of antibodies present among patients are shown in (Table 1). In our study, Ferozewala Tehsil appeared to be the most affected Tehsil in the Sheikhupura district (Table 2).

Table 1. Seroprevalence of confirmed dengue patients in Sheikhupura district by age group, sex and percentage of positivity for IgM and IgG during the year 2014-2017.

Characteristics	Patients, n (%)
Age group (n = 120)	
≤ 10 years	8 (6.6)
11-20 years	37 (30.8)
21-30 years	37 (30.8)
31-40 years	16 (13.4)
41-50 years	12 (10)
51-60 years	5 (4.2)
≥ 60 years	5 (4.2)
Ig positivity (n = 120)	
IgM	54 (45)
IgG	5 (4.2)
Both	61 (50.8)

Table 2. Tehsil-wise distribution of confirmed dengue cases in Sheikhupura district during the year 2014-17.

Tehsil name	Patients, n (%)
Ferozwala	62 (51.7)
Sheikhupura	31 (25.9)
Muridke	22 (18.3)
Sharaqpur	4 (3.3)
Safdarabad	1 (0.8)

Discussion

In the present study, the seroprevalence of IgM and IgG against DENV was examined using commercially available ELISA kits during dengue outbreaks in Sheikhupura. The emergence of DENV in 2014 had a high frequency (55%) of infections, which decreased markedly in the coming years, with scattered cases in areas having no previous reports of dengue cases. Here, our survey produced the baseline data on the spread of DENV and associated risk factors and concludes that the Sheikhupura and its environs are not safe from the risk of dengue infection.

We found the sero-prevalence of DENV IgM among febrile patients is higher (45%) than IgG (4.2%). The results are in agreement with findings described in Tanzania [15], Kenya [16], Eastern India [17], Brazil [18], Singapore [19], and Southern Pakistan [12], where DENV IgG was reported to be between 4.5% to 12%.

Figure 2. Graph showing year-wise DENV patients recorded during the years 2014-2017.

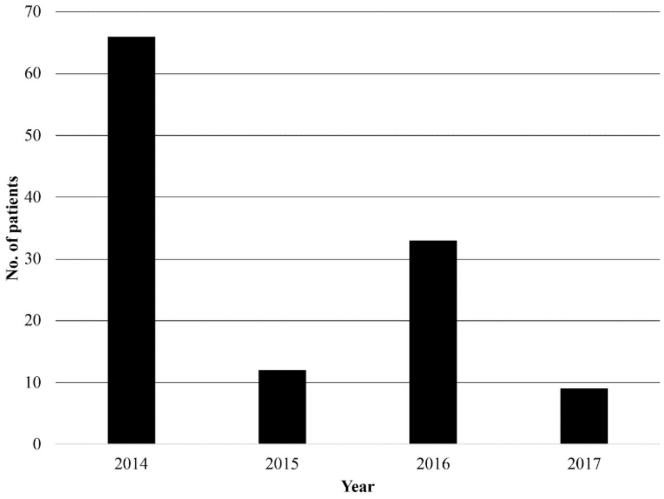
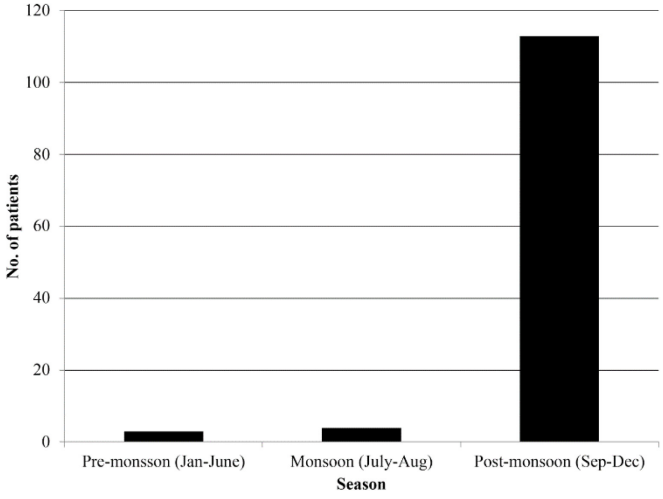


Figure 3. Graph showing season-wise DENV patients recorded during the study duration.



In contrast, the prevalence of anti-DENV IgG seropositivity in the present study was lower than the rates in South Ethiopia [20], Djibouti [21], and the northern province of Sudan [22], where it was reported to be between 20-24%.

The presence of dengue is associated with several factors. One of the key components of the transmission chain is the abundance of the vector. Vector density is related to weather characteristics, such as temperature, humidity, and rainfall [23]. The data obtained established the fact that most cases were recorded in the post-monsoon (September-December) period. However, fewer cases were noted in dry months also, illustrating that despite an epidemic profile, transmission persists in Sheikhupura in wet and dry seasons. There was also a significant correlation between DENV and environmental factors such as rainfall, temperature, and humidity, which is consistent with the conclusion in Myanmar [24], Brazil [18], Venezuela [25], India [17], China [26], Nepal [27], and Pakistan [12].

The study disclosed that dengue is predominant in men in Sheikhupura, and most cases appear in individuals aged between 11 and 30 years. These findings are persistent with gender-specific DENV cases published in Malaysia [28], Thailand [29], Singapore [19], Pakistan [12], Nicaragua [30], Japan, and Korea [31]. Our analysis found a significant relationship between gender, age, and exposure to dengue, which is related to the pattern of human occupation and cultural trends in the study area. The elevated prevalence among men is since male members travel and leave their houses for occupational purposes and are more prone to mosquito bites than females.

We reported 120 confirmed dengue cases from the study district. The data obtained presented that dengue expansion is advancing from the area where it emerged. The study anticipates that dengue in the Sheikhupura district may have spread from Ferozewala Tehsil, which neighbours the dengue-endemic Lahore city (Figure 1). From the findings of follow-up studies, it can be speculated that more tehsils of district Sheikhupura might experience much bigger outbreaks.

Although this is the first study reporting DENV infection in Sheikhupura, the study has several limitations. First, the ELISA method used in the study may have cross-reactivity with other Flaviviruses. But this serosurvey was conducted when no Zika virus or Japanese Encephalitis virus infection was reported in Sheikhupura. It justifies the ELISA results did not involve cross-reactions with other Flavivirus. Second, the study used anti-DENV IgG and IgM ELISA for confirmation and lacks information on dengue serotype prevalence. Therefore, further investigation is recommended that include other Arboviral infections in the differential diagnoses and perform molecular tests to explore additional serotyping details.

In conclusion, this study presented the prevalence of DENV IgG and IgM in Sheikhupura. It is a fact that when an emerging disease spreads into unfamiliar areas, it can go unrecognised. Therefore, the findings of this study have far-reaching implications for the planning and implementation of dengue prevention and control interventions in the area where dengue remains an emerging problem.

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Authors' contribution

Study conception and design: Dr Muhammad Uzair Mukhtar, Maria Mukhtar (Equal contributor) and Dr Naveed Iqbal. Data acquisition: Fatima Haq. Data analysis and interpretation: Dr Zeeshan Nawaz and Dr Muhammad Rashid. Draft revision: Dr Ali Arslan and Adil Bhatti.

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