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A Comprehensive Study of Dengue Epidemics and Persistence of Anti-Dengue Virus Antibodies in District Swat, Pakistan

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Keywords

Dengue · Immunoglobulin G · Antibodies · Swat · Pakistan

Abstract

Background: Dengue fever is one of the most common human arbovirus infections worldwide. In Pakistan, dengue initially became endemic in the big cities and then expanded to remote areas of the country. The current study reports the dengue epidemics, anti-DENV antibodies prevalence during the active and post-dengue infection, risk factors, disease symptoms, and spotting dengue infection densities in district Swat of Pakistan. ***Methods:*** Clinical signs and demographic data of dengue suspected individuals were collected at the time of screening through non-structural protein-1 antigen detection test during 2013–2015. Moreover, selected dengue confirmed individuals were screened for the presence of anti-dengue immunoglobulin (Ig) M and G during the active infection period and post-dengue infection. ***Results:*** A total of 8,770 individuals were infected with dengue in 2013 with 36 (0.41%) case fatalities, 307 in 2014 with no case fatality, and 13 in 2015 with no case fatality. The number of male and female cases were 6,139 and 2,631 in 2013, 183 and 124 in 2014, and only 10 and 3 in 2015, respectively. Among all the localities, Tehsil Babozai, an urban setting, reported the highest number of dengue patients during all the study years, that is, 7,673 (87.49% of the total cases) in 2013, 294 (95.76% of the total cases) in 2014, and 13 (100% cases) in 2015. Among 6 age groups, 21–30 years was found to be highly infected in 2013 (37.13% of all cases) and 2014 (33.55%). Furthermore, 1,231 (21.94% of all cases) had IgM antibodies and 71 (1.26%) had IgG antibodies in 2013, 78 (26% of all cases) had IgM antibodies and 7 (2.33%) had IgG antibodies in 2014, and only 4 (30.76%) patients had IgM and 0 (0%) had IgG antibodies in 2015. Furthermore, urban areas had the highest infection density in district Swat. The majority of the patients in rural areas had a traveling history to the urban areas before their illness. ***Conclusion:*** To sum up, male gender, young individuals, and those living in urban areas were at the greater risk of dengue infection.

Introduction

Dengue fever is one of the most common human arbovirus infections worldwide [1]. Dengue fever is caused by dengue virus (DENV), and female *Aedes* mosquitoes, es-

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pecially *Aedes aegypti*, are involved in the transmission of DENV from infected to susceptible individuals. Dengue fever has risen as a foremost public health concern. Dengue fever has become a threat to society and economic stability globally. Every year, the geographical extension and number of cases of dengue fever are increasing [2].

In the majority of DENV infections, 60–70% of cases are asymptomatic [3]. Nevertheless, symptomatic dengue infections mostly manifest as dengue fever, which is normally considered a self-limiting and non-fatal disease. The common symptoms include fever, headache, retro-orbital pain, nausea, muscle and joint pain, vomiting, and rashes [4, 5]. In some cases, the infection leads to a more severe life-threatening condition called dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [6]. DHF and DSS show increased plasma leakage, hemorrhagic symptoms, and high vascular permeability. Annually, more than 250,000 DHF cases are reported to the World Health Organization (WHO) with fatality rates of 1–5%, in which most deaths occur in infants [7–10].

It is very difficult to precisely diagnose dengue fever based only on clinical features because several dengue fever signs are similar to those of other infections, such as chikungunya, influenza, measles, and rickettsia. Diagnostic methods are primarily based on the detection of DENV-specific antibodies, viral genome sequences, and the viral antigens [11]. Moreover, both DHF and DSS can be diagnosed based on important clinical signs such as continuous high fever, hemoconcentration, hepatomegaly, thrombocytopenia, and shock [8].

DENV, the causative agent of the dengue fever, exists in 4 antigenically different forms/serotypes, that is, DENV-1, DENV-2, DENV-3, and DENV-4. It is a single-stranded enveloped RNA virus belonging to the genus *Flavivirus* within the family Flaviviridae [12]. The size of its RNA is 11.8 kb, and it encodes 10 proteins, out of which 3 are structural proteins such as membrane with its precursor (PrM), envelope (E), and capsid (C) proteins, while other 7 non-structural proteins are NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 [13, 14].

DENV transmits into the host body through the bite of *Aedes aegypti* or *Aedes albopictus* mosquito. Female *Aedes* attains the virus from infected individuals by feeding on their blood during the viremic phase of the disease and then transmits the acquired virus into a healthy susceptible person by biting [15, 16]. DENV transmission primarily occurs horizontally through the human-mosquito mode of transmission but vertical transmission can also occur. DENV can survive in eggs of the mosquito in unfavorable environmental conditions [17–19].

Dengue fever is prevalent in almost 100 countries worldwide and has expanded 30-fold in the last 50 years [2]. Worldwide, approximately 2.5 billion people are at risk of dengue infection [20]. According to the WHO, 50–100 million individuals are infected with dengue, of which 500,000 cases require hospitalization with 2.5% cases leading to mortality every year [21]. In 2013, 390 million people had dengue infections across the globe, which is 3 times higher than that estimated by the WHO in 2012 [22].

In Pakistan, the dengue outbreak was first reported in Punjab province in 1982 [23]. In 1994, the first DENV outbreak was confirmed serologically and virologically in Karachi [24]. Since then, the disease spread in the country in different years from small to severe dengue outbreaks with significant morbidities and mortalities in big cities like Karachi and Lahore [25–28]. In 2011, Lahore (the capital of Punjab province) was severely struck by DENV, where almost 23,000 people were hospitalized and 363 cases led to fatality because of DHF and DSS [27, 28]. Molecular studies showed that DENV-1, DENV-2, and DENV-4 serotypes were involved in the huge outbreak in Lahore in 2011 [29].

In 2013, just 2 years after the severe dengue fever outbreak in Lahore, a huge dengue outbreak struck district Swat, Khyber Pakhtunkhwa. District Swat, located in the North, is 600 km away from Lahore. The district has a temperate climate (annual average rainfall exceeds 1,000 mm and average temperature recorded is 18°C) compared to Lahore, where annual average rainfall measures at 430–767 mm and mean annual temperature recorded is 28–31°C [30]. Thousands of individuals got infected with dengue in Swat and 36 cases of death occurred according to the official report of the district health office (DHO) in 2013. In 2014, a moderate dengue outbreak occurred again in the district, followed by a minor dengue outbreak with few cases in 2015. The current study was carried out to provide a comprehensive insight into the dengue outbreaks in district Swat during 2013–2015.

Materials and Methods

Study Area

All the clinical and demographic data and blood samples from dengue patients for NS1 antigen detection test and antibodies prevalence during infection and post-infection were collected from district Swat. District Swat is a beautiful valley located between 34° 34″ and 35° 55″ North latitudes and 72° 08″ and 72° 50″ East longitudes in Malakand division, Khyber Pakhtunkhwa Province of Pakistan. In summer, the temperature rises above 37°C with relatively high humidity in the monsoon rain season. The human

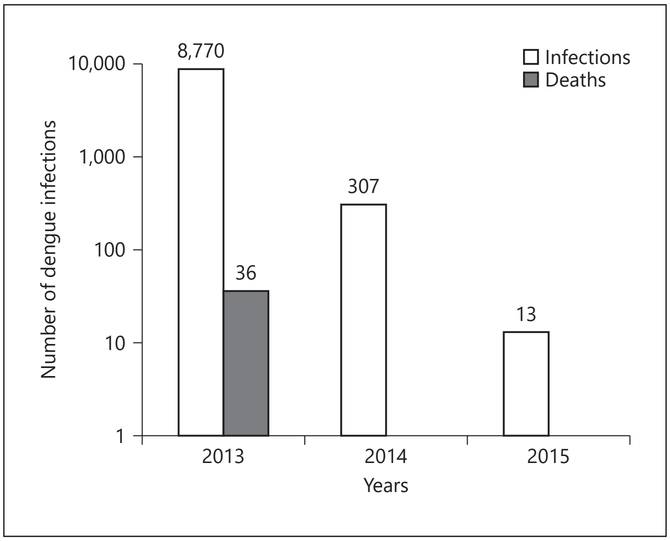


Fig. 1. Dengue infections and deaths in district Swat in 2013, 2014, and 2015.

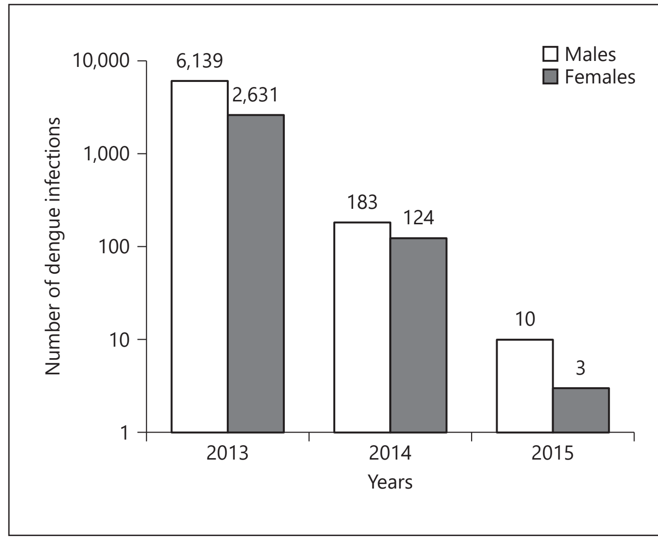


Fig. 2. Gender-wise distribution of dengue cases in district Swat in 2013, 2014, and 2015.

population in District Swat is estimated as 2,161,000. In 2013, thousands of dengue cases were reported from the district in the summer season when the temperature and relative humidity were higher in the area.. Dengue epidemics continued to occur in the next 2 years with a medium and small level outbreaks in 2014 and 2015, respectively.

Sampling and Data Collection during 2013, 2014, and 2015 Dengue Outbreaks in District Swat

During the 2013 outbreak, the infection was confirmed mainly based on the NS1 antigen detection test in patients suspected for dengue. Clinical and demographic data of dengue patients were collected at the time of initial screening through the NS1 antigen test along with information regarding gender, residence location, age, travel history, and clinical signs. Moreover, dengue confirmed individuals were tested for the presence of anti-dengue antibodies (immunoglobulin [Ig] M and IgG antibodies) during the infection period and post-dengue infection (several months after the recovery).

Screening for NS1 Antigen and Dengue-Specific IgM and IgG Antibodies

Blood sample (5 mL) was collected from each dengue patient during the outbreak and then centrifuged at 3,000 rpm for 10 min to collect serum. The labeled serum samples were then tested for the presence of NS1 antigen by SD Dengue Duo rapid strips (Standard Diagnostics Inc., Korea) and specific anti-dengue (IgM and IgG) antibodies. In the current study, dengue recovered individuals (n = 100) were also tested for the presence of dengue-specific IgM and IgG antibodies 2 years post-infection using SD Dengue Duo rapid test for NS1 antigen detection.

Geographic Information System Mapping

For this study, a geographic information system (GIS) map of district Swat was drawn using the software Q GIS 2015 for the analysis and spotting of dengue outbreak infection densities in localities of district Swat for the years 2013, 2014, and 2015.

Statistical Analysis

A χ^2 test was used to compare different parameters/variables, where p value <0.05 was considered significant. The analysis was carried out using SPSS 20.

Results

Epidemiological Study of Dengue Outbreaks in Swat

Dengue Infections and Deaths in District Swat

In 2013, the first severe major dengue outbreak occurred in Swat. A total of 8,770 individuals suffered from dengue fever with 36 (0.41%) fatalities. The dengue infections then sharply declined in 2014 and to an almost negligible level in 2015. Only 307 cases were reported in 2014 and 13 cases were confirmed in 2015 with no fatalities during both the years (Fig. 1).

Gender-Based Distribution of Dengue in District Swat

Dengue infection was significantly higher in males than in females ($p < 0.05$). The results showed that 6,139 (70%) males and 2,631 (30%) females were infected with DENV in 2013. In 2014, 183 (59.60%) males and 124 (40.39%) females were infected, while 10 (76.92%) males and 3 (23.07%) females had dengue infection in 2015 (Fig. 2).

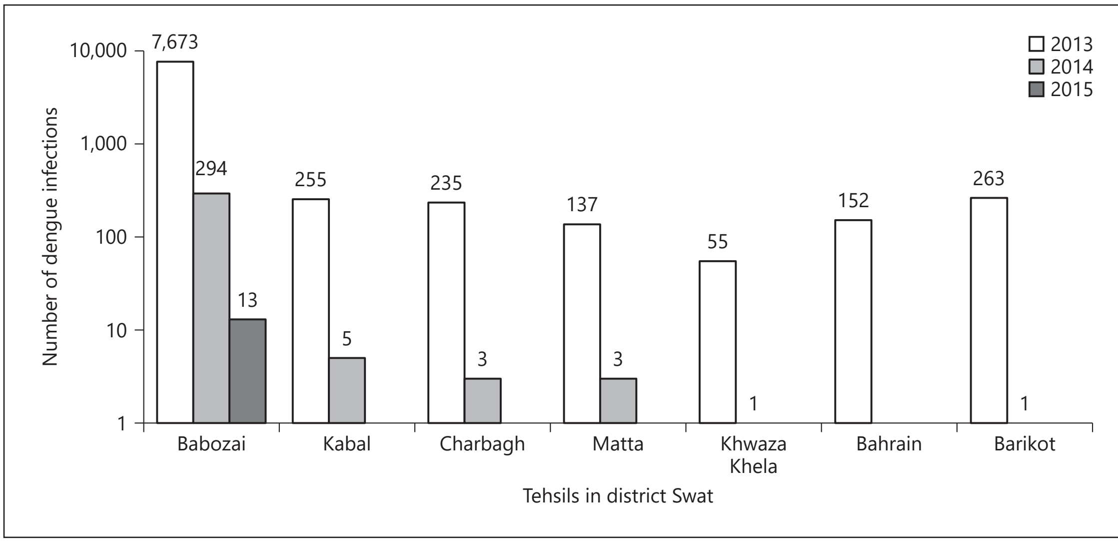


Fig. 3. Tehsil-wise distribution of dengue cases in district Swat in 2013, 2014, and 2015.

Tehsil-Wise Distribution of Dengue Cases in District Swat

Tehsil Babozai (urban area) was highly affected due to dengue fever compared with any other Tehsil in 2013 and in subsequent minor outbreaks in the following 2 years. In 2013, 7,673 individuals from Tehsil Babozai were infected with DENV, which accounts for the highest proportion (87.49%) of the total number of cases in the district Swat in that year. In the same year, the number of cases in the remaining Tehsils, that is, Barikot, Kabal, Charbagh, Bahrain, Matta, and Khwazakhela was 263 (2.99%), 255 (2.90%), 235 (2.67%), 152 (1.73%), 137 (1.56%), and 55 (0.62%), respectively. In 2014, the number of cases was again highest in Tehsil Babozai (95.76%; $n = 294$), while only 5 (1.62%) cases in Tehsil Kabal, followed by Matta ($n = 3$), Charbagh ($n = 3$), Khwazakhela ($n = 1$), and Barikot ($n = 1$). Similarly, in 2015, dengue cases occurred on a very small level with only 13 cases, all of which belonged to Tehsil Babozai (Fig. 3).

Age-Wise Distribution of Dengue Cases in District Swat

The current study revealed that individuals in the age group 21–30 years were the most infected with dengue, followed by age groups 11–20 and 31–40 years. In 2013, individuals in the age group 21–30 years had the highest number of infections ($n = 3,257$; 37.13%), followed by age groups 11–20 years ($n = 2,224$; 25.35%) and 31–40 years ($n = 1,641$; 18.71%). Children in the age group 0–10 years had the least dengue infections ($n = 278$; 3.17%) and elderly people >50 years of age were the second least infected individuals ($n = 592$; 6.75%). In 2014, the highest number of dengue cases occurred in the individuals in the age group 21–30 years ($n = 103$; 33.55%), followed by age groups 11–20 years ($n = 83$; 27.03%) and 31–40 years ($n = 53$; 17.26%). Children in the age group 0–10 years and elders in the age group >50 years had only 8 (2.60%) and 23 (7.49%) dengue infections, respectively, in 2014. Of the total 13 dengue cases in 2015, 6 (46.15%) were in the age group 31–40 years, followed by 2 cases (15.38%) each in age groups 0–10, 11–20, and 21–30 years. No dengue infection was recorded in individuals aged >50 years in the year 2015 (Fig. 4).

Persistence of Anti-DENV IgM and IgG in Dengue-Infected and Recovered Individuals

In 2013, a total of 5,610 dengue patients were screened for the presence of IgM and IgG after DENV confirmation by NS1 antigen detection test. Of these, 1,231 (21.94%) individuals were found positive for IgM and 71 (1.26%) for IgG. In the year 2014, among 300 confirmed dengue cases, 78 (26%) and 7 (2.33%) were found to have IgM and IgG, respectively. In 2015, only 4 (30.76%) cases were found to have IgM and no individual was found positive for IgG antibodies. The results showed that the prevalence of IgM and IgG was not statistically associated ($p > 0.05$) with the dengue prevalence in 2013, 2014, and 2015. Furthermore, the post-infection study of confirmed

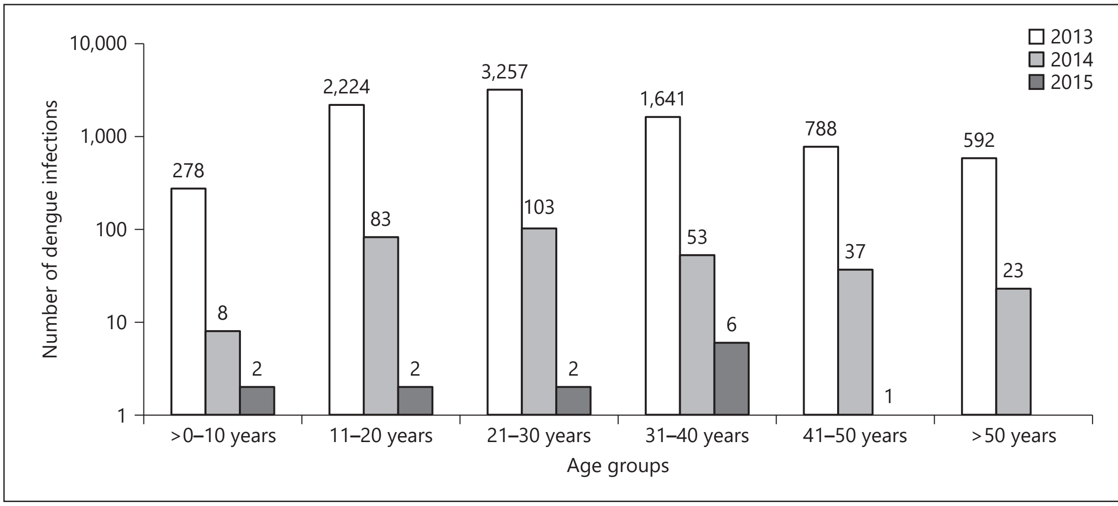


Fig. 4. Age-wise distribution of dengue cases in district Swat in 2014.

cases (several months after the infection) revealed that among 100 recovered individuals, only 35 individuals had anti-DENV IgG but none of the individuals had anti-DENV IgM.

Traveling Contribution to Dengue Transmission in Swat

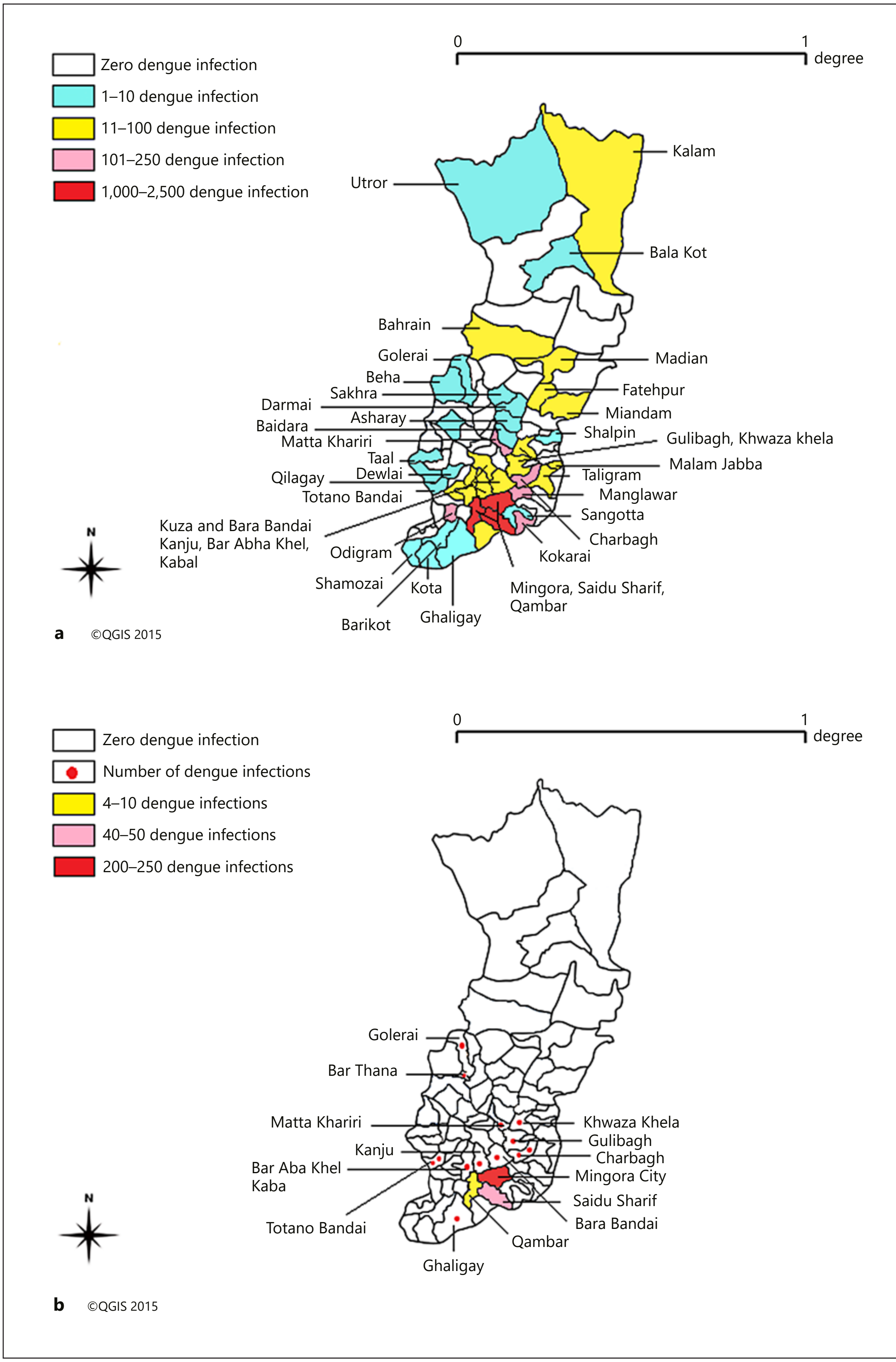
In the current study, confirmed cases in the rural areas of district Swat were analyzed to deduce the possible factors contributing to dengue transmission. The study showed that individuals suffered from dengue infection in all the 6 tehsils outside of the urban region had traveling history to the urban area, that is, Mingora city (Tehsil Babozai) before dengue infection in district Swat. In Tehsil Kabal, 97.25% (n = 248) of the infected individuals traveled to Mingora city, followed by Khwazakhela (96.36%, n = 53), Bahrain (95.39%, n = 145), Matta (94.89%, n = 130), Charbagh (94.04%, n = 221), and Barikot (87.83%, n = 231) within 2013. In 2014, 100% of the dengue cases in each rural tehsils were linked with traveling to Mingora city, whereas no infection was confirmed in any other tehsil. The traveling history of dengue patients to Mingora city before dengue infection was significantly associated ($p < 0.05$) with the prevalence of dengue in all the tehsils in 2013 and 2014.

Clinical Signs of Dengue-Infected Patients in District Swat

A total of 1,125 dengue suspected cases were analyzed to observe the clinical manifestations in the patients with disease. Among the suspected patients, 799 (71.02%) were found positive, whereas the rest 326 (28.97%) were negative for the NS1 antigen. In the dengue-positive patients, the clinical signs were present with high frequency. Fever was experienced by 790 (98.87%), followed by a headache by 788 (98.62%), skin rash by 571 (71.46%), pain behind the eyes by 512 (64.08%), joint pain by 501 (62.70%), fatigue by 462 (57.82%), and nausea was manifested by 421 (52.69%) individuals. However, in dengue-negative patients, the dengue-related clinical symptoms were present with low frequency, such that fever was observed in 315 (96.62%), headache in 270 (82.82%), skin rash in 151 (46.31%), pain behind the eyes in 102 (31.28%), joint pain in 101 (30.98%), fatigue in 91 (27.91%), and nausea in 90 (27.60%) individuals.

Dengue Infections Density Mapping through GIS Map in District Swat

In 2013, according to the GIS mapping, urban areas such as Mingora city, Saidu Sharif, and Qambar were greatly affected by dengue epidemics, where the density of infections ranged from 1,000 to 2,500 infections in each area, as shown by red color in the map (Fig. 5). The disease was also present in the vicinity of urban areas with a great density of infections mapped with pink color ranging from 101 to 250 infections in each area, that is, Manglawar, Odigram, Charbagh, and Kokarai. Some of the areas shown in yellow color in the vicinity of the urban region and far distant remote areas such as Kanju, Kabal, Bar Aba Khel, Kuza Bandai, Bara Bandai, Gulibagh,



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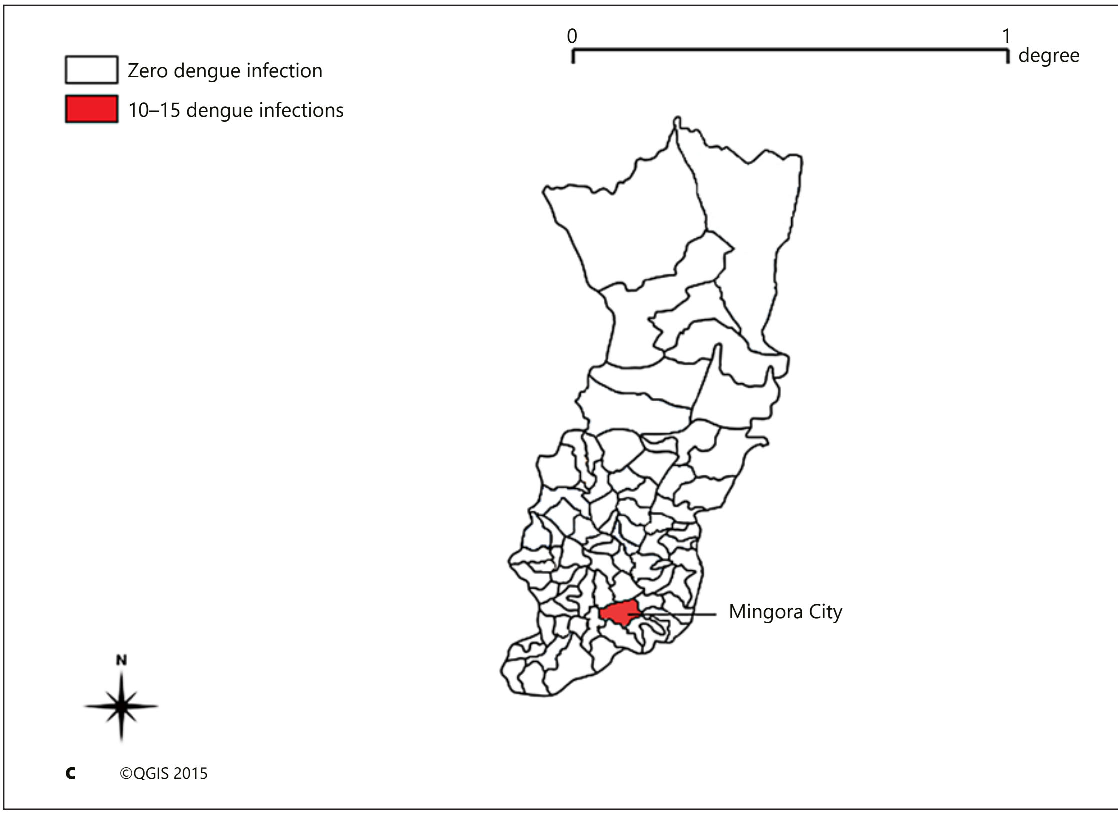

Fig. 5. Union council-wise density of dengue cases in Swat in 2013 (**a**), 2014 (**b**), and 2015 (**c**).

Khwazakhela, Miandam, Fatehpur, Bahrain, and Kalam had the density of infections from 11 to 100 infections. The density of infections remained very less in the remote areas such as Utror, Balakot, Golerai, Beha, Sakhra, Baidara, Taal, Qilagay, Dewlai, Totano Bandai, Shamozai, Kota, and Ghaligay only at 1–10 infections per area (Fig. 5).

In 2014, however, the frequency of dengue epidemics was 25–30 times lesser than that of the 2013 infection. In 2014, Mingora city (urban region of district Swat) witnessed the highest number of dengue infections than any other area of district Swat. In Mingora city, GIS mapping showed the highest number of infections with 200–250 cases as shown in red color in the map (Fig. 5b). Saidu Sharif area had the second highest frequency of dengue infections with 40–50 cases (shown in pink), followed by Qambar area (yellow color) having 4–10 infections. Some areas of the district such as Kanju, Kabal, Totano Bandai, Barthana, Gwalerai, Khwazakhhela, Matta Khariri, Guligagh, Charbagh, and Ghaligay had only 1–2 infections, while the rest of the areas in Swat witnessed no dengue case during the year 2014. In 2015, Mingora city witnessed all the dengue infections, with no documented case in rest of the district (Fig. 5c). GIS mapping showed a clear trend of dengue declining from the major dengue epidemics in the year 2013 to minor cases in 2015.

Discussion

The current study highlights the comprehensive picture of dengue epidemics starting from the major and severe outbreak in 2013 to the few dengue cases in 2015 in district Swat, Khyber Pakhtunkhwa Province of Pakistan. Dengue is present in Pakistan since 1982 [3] with variation in severity of the disease in different parts of the country. In 2013, a major dengue outbreak reported for the first time with a total of 8,770 dengue infections and 36 (0.41%) deaths in district Swat. This epidemic was after a major outbreak during 2011–2012 in Lahore, which resulted in 350 deaths (1.61% mortality rate) [31]. Although the fatality rate in district Swat was lower than that in Lahore, it compares with fatalities in other Asian countries, where case fatality rate of the disease ranged from 0.09 to 0.33% [32]. The deaths due to outbreak in 2013, when analyzed, revealed that elderly men were mostly the victim, which might be because of their exposure and vulnerability due to age-related diseases [32]. However, district Swat had a lower fatality rate than Lahore, which may be due to many factors including difference in climate, topography, and population density. Swat lies in the mountains, and thus, its landscape drastically reduces the chances of water stagnation. This ultimately leads to an unfavorable environment for the mosquito to reproduce. In contrast, Lahore is hotter and more humid than Swat, which increases the chance of survival of mosquitoes for longer time in the former area.

Dengue epidemics decreased sharply to 307 and 13 infections from 2013 to 2014 with no mortality in 2015 (Fig. 1). The decreasing epidemiological trend over the years was also observed by Khan et al. [33]. Studies suggested that DENV-2 and DENV-3 were dominantly circulating in the area linked with climate change, traveling of an infected human, socioeconomic and sociodemographic factors and urbanization. The higher incidence of dengue could be because of the substantial viral load in moving population and transportation of *Aedes* mosquitoes and their larvae from dengue-endemic regions of Pakistan, where the disease was already diagnosed in the past years [25, 33–36]. The same phenomenon could have possibly played a role in massive dengue transmission primarily into the urban region (Mingora city) from the other endemic areas of Pakistan such as Karachi, Lahore, and Rawalpindi. In 2011, a more severe dengue attack erupted with almost double the number of infections and a 4-fold mortality proportion than the 2013 outbreak of district Swat, which caused 17,493 infections with 290 (1.65%) fatal cases in Lahore, Punjab, Pakistan. It is suggested that traveling, transport, and extensive trade of different commodities such as old tires and other raw materials contaminated with dengue mosquito eggs from Lahore (Punjab province), Karachi (Sindh province), and Peshawar (Khyber Pakhtunkhwa) facilitated the dengue emerging in district Swat [27]. Khan et al. [31] demonstrated that 37% of the *A. aegypti* was found in stagnant water in drums, followed by drinking water tanks (23%). This explains the reason behind the massive outbreaks in 2013. They also associated the dengue outbreak in Swat with the other species of the vector that is, *A. albopictus* in addition to the main vector, *A. aegypti.* The predominant reason seems to be the fact that as the dengue outbreak started near the monsoon season in Swat (in which pre-monsoon rains start), the monsoon rains might have played an important role in spreading dengue across the district [37].

In the current study, it has been observed that human males are comparatively more infected than human females by dengue. These results are in accordance with the published studies, where male infection ratios were higher than those of females [31, 33, 38]. A high proportion of dengue in males compared to females could be attributed to the fact that females, in the study area, culturally covers most body parts such as arms, face, and feet and are thus less exposed to dengue carrier mosquitoes. Second, women were not actively involved in traveling to cities compared to males, hence they have a minimum probability of being infected with dengue in Swat, Khyber Pakhtunkhwa, Pakistan.

Dengue is usually observed as an urban disease so far and this fact is also partially applicable to populated towns in rural regions. The current study showed that 87.49% (n = 7,673) dengue infections occurred in the urban area (Tehsil Babozai) of district Swat in 2013 and the same trend continued with a higher number of cases in the urban area in 2014 and 2015 (Fig. 3). The reason for this might be the fact that urban areas in remote locations of developing countries like Pakistan are exposed to issues such as unplanned cities development, uncontrolled human population growth, poor drainage system, lack of proper waste management, trade, traveling, poor sanitation, and substandard housing. This might have led to greater reproduction and vast dispersion of dengue mosquitoes in the urban area of the District Swat, thus putting the inhabitants at a greater risk of dengue infections. The study findings were consistent with Ali et al. [39] where lower proportions of anti-dengue antibodies (IgM 23% and IgG 13.41%) were detected in rural areas compared to urban areas (IgM 41.13% and IgG 27.42%). Furthermore, the study was found in concordance with the findings of Hayes et al. [40] who observed that dengue prevalence was higher as 66% of the urban population compared to 26% of the rural population.

Furthermore, the high incidence of dengue infection in the young age group 20–30 years is harmonious with previous studies conducted in other dengue epidemic localities of Khyber Pakhtunkhwa, where the age group 20–30 (35.29%) years was found mostly affected by dengue followed by age groups with 11–20 (21.89%) and 31–40 (18.95%) years, whereas children of age 0–10 years and elderly people aged >50 years were least infected with dengue at 4.08 and 11.60%, respectively [39]. Chaudhry and colleagues [32] also reported a high proportion (50%)

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of dengue cases in the male age group 21–40 years compared to other age groups, highlight the fact that while children may be more exposed to the virus because they usually sleep exposed in the summer season [31], adults due to prior exposure and other age-related physiological parameters are more likely to have clinical dengue than children [32]. The high proportion of dengue cases in young age groups may be because of their active social involvement and thus active traveling compared to the elderly and children who are less active socially and less in traveling and movement. This might have made the young age group exposed more to DENV. The study findings were further supported by other comprehensive studies [31, 33, 41].

The results of antibodies (IgM and IgG) seroprevalence in our study were that found in a study carried out in district Swat [38], where the proportion of dengue-specific antibodies was 24.50% ($n = 1{,}470$) in 2013. Normally, IgM antibodies are detectable in 3–5 days after the illness and then decline in 2–3 months after the dengue illness, while IgG antibodies are detectable after a week of the illness and last to several months or maybe present lifelong [42, 43]. The prevalence of dengue-specific antibodies in the current study is also found much lower than that in an epidemiological study conducted in Lahore, where the prevalence of IgM was 48.66% ($n = 166$) and IgG was 39.5% ($n = 79$) [44].

In the current study, it was found that IgG antibodies were present after several months in the individuals ($n = 35$) who recovered after dengue infection, while no IgM antibodies were found in the post-dengue infections. This has been confirmed through various studies done on decades-old dengue cases [45–47]. These studies have shown that anti-dengue IgG can be found even after 40 years of post-infection. In terms of the IgM antibodies, the present study was found consistent with other such reports [42, 48]. These studies revealed that IgM antibodies are detectable 3–5 days after the onset of illness and decline to undetectable levels after 2–3 months, whereas the IgG antibodies are detectable after the first week of illness in serum and up to several months of infection and even could be present lifelong. Studies have shown that DENV-3 serotype was most prevalent in Swat during the years 2013, 2014, and 2015, followed by DENV-2 during these years. For instance, Khan et al. [33] have reported that out of 100 blood samples collected in 2013, 55 samples were positive for DENV-3 and 40 showed the presence of DENV-2, while the rest showed mixed infection by these 2 serotypes. A similar pattern was observed in 2014 (65 for DENV-3 and 34 for DENV-2 out of 100 samples) and 2015 (12 for DENV-3 and 8 for DENV-2 out of 20 samples). Although serotype of the DENV was not determined in the present study, the lower mortality rate in 2014 outbreak compared to the 2013 outbreak and no mortality in the 2015 outbreak in the study area show that the same serotype(s) of DENV was involved in these infections and excluding the possibility of secondary DENV infections in the study area.

In our study, the frequency of clinical signs was very high in the dengue-positive cases compared to negative cases, which was highly consistent with the previous study [39], where clinical signs such as fever, headache, joint pain, fatigue, and skin rashes were statistically linked with dengue.

Traveling to dengue-endemic areas has always remained a significant factor in dengue transmission in different countries. In the present study, it was proposed that Mingora city (in Tehsil Babozai) is already a potential endemic site, and in this regard, the infected people outside Mingora city were evaluated for their traveling history to Mingora city before infection. Once the disease became endemic in the urban region, then it was dispersed proposedly into other far distant tehsils through traveling and trade in district Swat. Every single person infected with dengue could become a DENV reservoir for the uninfected mosquitoes, and then, it develops a cascade of dengue epidemics in the region. An entomological study has already confirmed the widespread distribution of dengue mosquitoes and their larvae in the urban region of district Swat in 2013 [31, 33]. The current study showed that traveling to urban areas is statistically associated with dengue cases in rural non-endemic areas in district Swat. The observation suggests that individuals traveling to urban regions (dengue-endemic cities) are at a higher risk of being infected with dengue. Current findings agree with many other studies on the topic where the traveling was significantly linked with dengue [31, 37, 39, 49, 50].

GIS mapping showed the density of dengue infections was far higher in urban areas (Mingora, Saidu Sharif, and Qambar) compared to the very low density of dengue infections in rural areas, as shown in Figure 5. The present GIS mapping had very close similarities with the other dengue geographic-based studies conducted in district Swat [31, 38]. Several other studies showed urban and populated areas are at a higher risk of dengue and had higher dengue prevalence compared to rural and least populated areas [39, 40].

Conclusion

The current study provides a comprehensive insight into dengue epidemiology, risk factors, antibodies prevalence during infection and post-dengue infection, and dengue risk zones in district Swat. The current study showed that male gender and young individuals are at a greater risk of dengue infection. Dengue infections were highly prevalent in the urban region compared to rural regions of district Swat. People traveling to the urban regions and cities during the dengue infection season could acquire dengue infection with a high probability compared with the one not traveling endemic cities during dengue infection season. The study revealed during the period of active dengue infection, IgM antibodies are more prevalent than IgG antibodies and IgG antibodies are presumably present at very low titer, while in the post-dengue period (dengue recovered individuals), IgG antibodies are more prevalent and IgM antibodies were not detected. In dengue-positive patients, clinical signs such as fever and headache among others are present in majority of the patients. Moreover, it is very important to further characterize the serotypes and genotypes of DENV responsible for the disease outbreaks in district Swat.

Acknowledgement

We acknowledge the role of the District Health Officer, Swat, in provision of useful information on dengue in the area.

Statement of Ethics

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. Informed consent was obtained from the enrolled subjects. No ethical approval was required as only standard samples were obtained from suspect cases of dengue for diagnostic purpose. This exemption was granted by the "Departmental Bio-ethical Committee" of the Department of Biotechnology, University of Malakand, vide reference number UoM/Biotech/DBC/0012/13.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

S.M.J. designed the research project. N.A. conducted the experiments. N.A. and S.M.J. wrote the manuscript. S.M.J. and T.K. reviewed the manuscript and revised the figures.

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