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Sero-epidemiological Identification of Dengue Virus in Individuals at District Shangla, Khyber Pakhtunkhwa, Pakistan

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Abstract

The current study was performed to assess the serological aspects of dengue infected patients during 2018 at District Shangla, Khyber Pakhtunkhwa, Pakistan. During 2018, a total of 1447 visited these hospitals. Blood samples from the patients at pathology laboratory. The data was taken from hospitals for the span of 5 months (August to December). A separate form was used to notice the gender and history of the individuals. The blood samples collected were screened for Dengue infection with the help of BIOLINE Dengue NS 1 Ag+Ab Combo Test. A total of 1447 blood samples were screened. Among them 554 were found positive for dengue fever whose acquired percentage was (38.28%), while 893 (61.71%) samples were determined with the negative outcome. With respects to patients age groups, there were 271 patients found positive with Dengue infection ages of 15 to 35 years with a percentage of (48.91%) which constitutes the majority of majority dengue infected patients. In the month wise estimation of the Dengue severity in the patients, a major outbreak was identified in September and October because of the rainy and post rainy season. It is concluded that approaches including biological, environmental, medicinal, as well as the educational awareness should be taken to overcome the infection. There is also a need of making teams for

investigating the real cause and impacts of the dengue fever on the people of the district.

Keywords: Dengue fever; s-Virus; Prevalence; Shangla; Pakistan

Introduction

Dengue fever is the most prevalent vector born disease that causes persistent and significant health hazards all over the world. It outspreads mostly in the tropical and subtropical expanses across the globe. In the few decades, dengue virus (DENV) infection has been increased by thirty-fold due to its spreading into other countries and from urban to rural areas. More than 40 billion people are infected annually and over 50% of the world's population is now at risk of infection due to living in a DENV endemic area [1,2]. The DENV belongs to flaviviruses family having four distinct serotypes (DENV-1 to DENV-4) transmitted by Aedes aegypti and Aedes albopictus. Aedes aegypti is a domestic mosquito that preferably feeds on humans. This is a highly urbanized mosquito; breeds in collected rainwater or in water stored for domestic purposes [3,4]. Since 1992, Pakistan has experienced several dengue fever (DF) outbreaks in 3-5-year cycles. The very first confirmed outbreak was reported in Karachi city of Pakistan in 1994 in which two DENV serotypes, DENV-1 and DENV-2 were identified [5]. The DENV-3 serotype was also reported in

Karachi during the 2005-06 outbreaks [6]. In 2007-09 outbreaks in Lahore, DENV-2 and DENV-3 were found the predominant serotypes [7]. In 2011 and 2013, two devastating outbreaks of DF were reported with 4000 deaths in Lahore (Punjab province) and Swat (Khyber Pakhtunkhwa province) respectively [8]. In 2017, in a study from Khyber Pakhtunkhwa (KPK) documented 24,938 DF cases that occurred in 15 different districts of the province [9].

In this study, we have focused on Shangla district of KPK to whom less attention was given during the epidemics. We also aimed to investigate the disease severity, clinical features, laboratory findings and outcome of serologically confirmed cases of DF in patients during the recent dengue epidemic.

Materials and Methods

Study site

Shangla district is situated in Malakand division of the province Khyber Pakhtunkhwa in Pakistan. The total area of the district is 1,586 square kilometres. During 2018, dengue severity was recorded in two main health centres of the district Shangla DHQ Alpuri and THQ Besham.

Data collection

Data of the patients was obtained from the above-mentioned hospitals with the permission of the Medical superintendents. A total of 1,447 suspects were screened for dengue. The investigation was recorded based on month, age category and type of diagnostic test performed.

Diagnostics tests for dengue fever examination

For the diagnosis of dengue fever, BIOLINE dengue NS1 Ag +Ab Combo Test (Standard Diagnostics, INC, Korea) were used.

Basic steps of the test: BIOLINE Dengue NS 1 Ag+Ab Combo Test is a rapid test designed to concurrently examine and differentiate both the antibodies, i.e. IgG and IgM against the dengue virus in human plasma, serum, or whole blood. A window of Dengue NS1 Ag kit is labelled with two pre-coated lines T for NS 1 Ag test Line and C for Control Line. The test line presented for sample addition and the upper side control line supposed for limiting the sample inside to the device. These two lines in the window do not turn visible prior to sample addition. All three types of specimens (plasma, serum or whole blood) can be used for diagnosing in the device to result out the Dengue virus antigen NS1, IgG and IgM with intense sensitivity and particularity. The following tests can also be performed to identify all four Dengue serotypes by using recombinant dengue envelope proteins mixture. All three pre-coated lines on the surface of the device, "G" (Dengue IgG Test Line), "M" (Dengue IgM Test Line) and "C" (Control Line) performing their specific limits. With respect to their working procedure, the "Control Line" is used for sample controlling.

Upon sample addition to the device. If 'NS1' IgG/IgM, in the result window is present, a purple "G" and "M" lines will be

visible. If Dengue virus in the sample is not present, then colour arrival will not happen on the result window. When the sample is added to the well of the device, a reaction triggers between anti-dengue IgGs and IgMs in the sample and recombinant dengue virus envelope proteins which forms antigen-antibody complex. This complex adapts capillary movement along the length of the device. A specific colour line will be appeared if relevant anti-IgG and anti-IgM antibodies of human immobilized in two test lines and make a complement through the test device.

Dengue NS-1 Ag: In this test, firstly three drops (about 100 µl) of the plasma, serum or whole blood are added into the sample well (S). After 15-20 minutes, results of the test are found when a single colour line appears within the result window which confirms a positive case. When two colour lines appeared i.e. T band and C line on the side of the result window, it shows a negative result.

Dengue IgG/IgM: A sample of 10 µl of plasma, serum or whole blood is added into sample well (S) using capillary pipette. Then assay diluents were added into the well in the form of four drops. After 20 minutes, result appeared. Positive results indicate the primary, secondary, late primary, or early secondary infection of the dengue fever.

IgM/IgG positive: When two lines "C and M" appear in the result window, it shows that IgM is positive. If "M" line is weak, it indicates primary Dengue infection. Two lines "C" and "M" in the result window indicate that IgM is positive. Although a weaker "G" line depicts secondary infection.

IgG and IgM positive: When all the "C", "M" and "G" lines appear in the result window, it explains that IgM and IgG are positive and the infection is late primary or early secondary dengue fever. A single line "C" at right direction of the device window reflect the negative result. There is no control ("C") line in the window tell unwell result.

Results

The present study was outlined from August to December to examine the Age-wise and Month-wise severity of the dengue infection in the residents of district Shangla (KP, Pakistan). Laboratory tests were conducted in two main Government hospitals DHQ Hospital (Alpuri) and, THQ Hospital (Besham). Of the total 1,447 patients screened, 554 patients were found positive for DF and 893 were negative cases.

Outcomes based on different diagnostic assays

Overall, 275 (49.63%) patients found positive for IgM, 136 (24.54%) resulted positive for IgG and 91 (16.42%) resulted for NS1 and 7 (1.26%) positive cases obtained for NS1+IgG. Similarly, 9 (1.62%) patients were positive for NS1+IgG+IgM and 36 (6.49%) patients were positive for IgG+IgM. Total male patients diagnosed were 379 (68.41%) while female patients were 175 (31.58%). In the total of 275 individuals diagnosed for IgM, age wise estimation remained quite different. Out of 275 (49.63%) positive cases, 69 patients (12.45%) were found at the age ranged from 1 to 15 years, 139 (25.09%) in the age

group 15 to 35 years, while in age category 35 to 60 years. 67 (12.09%) patients were observed. The total number of positive cases for IgG were 136 (24.54%). Based on different age groups, 41 (7.40%), 60 (10.83%), 35 (6.31%) cases were recorded for 0 to 15 years, 15 to 35 years and 35 to 60 years respectively. Similarly, in the total 91(16.42%) positive patients with NS1, the number of patients for different age groups, i.e.; 0 to 15 years, 15 to 35 years and 35 to 60 years were 26 (4.69%), 43 (7.76%) and 22(3.97%) respectively. And the overall result for NS1+IgG, only 7 patients (1.26%) were found positive. In which the age-wise prevalence was, patients with ages 0-15 years was 1 (0.18%) and for ages 15-35 years were 6 (1.08%) patients. With ages 35-60 years, no positive patients were found in this age group. While the total obtained result from dengue patients for NS1+IgG+IgM, was (1.62%) and total positive patients were 9, while the obtained results for age-wise prevalence were 2 (0.36%), 7 (1.26%) and 0 for the above-mentioned age groups. And the total result obtained for both IgG+IgM was (6.49%) with 36 positive patients. According to their age-wise results, patients with ages 0-15 years were 11 (1.98%), while patients of age 15-35 years were 16 (2.88%). And patients with ages 35-60 years had 9 (1.62%) individuals who were reported positive, as shown in **Tables 1 and 2**.

Table 1: Gender and anti-dengue antibodies wise prevalence of dengue positive individuals.

Anti-dengue Antibodies	Male Patients	Female Patients	Total Patients	Percentage%
IgM	192	83	275	49.63%
IgG	88	48	136	24.54%
NS1	62	29	91	16.42%
NS1+IgG	6	1	7	1.26%
NS1+IgG+IgM	5	4	9	1.62%
IgG+IgM	26	10	36	6.49%

Table 2: Age-wise prevalence of dengue positive individuals.

Anti-dengue Antibodies	0-15 Years	15-35 Years	35-60 Years	Percentage%
IgM	69	139	67	49.63%
IgG	41	60	35	24.54%
NS1	26	43	22	16.42%
NS1+IgG	1	6	0	1.26%
NS1+IgG+IgM	2	7	0	1.62%
IgG+IgM	11	16	9	6.49%

District Headquarter hospital (DHQ, Alpuri) results

In DHQ hospital, a total of 485 tests were conducted in which 164 (33.81%) were obtained positive for dengue infection and 321 (66.18%) were found negative. Among these, 113 (68.90%) were male patients and 59 (35.97%) were

female patients. According to the overall diagnosed result of the patients, about 77 patients with percentage of (46.95%) found positive for IgM and 38 (23.17%) patients found positive with IgG. Likewise, 35 (21.34%) patient's blood samples were found positive for NS1. And 2 (1.21%) individuals were NS1+IgG +IgM positive. Further, 10 (6.09%) patients were found positive for both IgG+IgM, (Anti-Dengue-Antibodies) (**Table 3**).

Table 3: Outcomes from DHQ, Alpuri.

Anti-dengue Antibodies	Patients Number	Percentage%
IgM	77	49.95%
IgG	38	23.17%
NS1	35	21.34%
NS1+IgG	2	1.21%
NS1+IgG+IgM	2	1.21%
IgG+IgM	10	6.09%

Tehsil headquarter hospital (THQ, Besham) results

In THQ Besham, overall, 962 blood samples were examined for DF in which 390 (40.54%) were positive and 572 (59.45%) were negative. Out of 390 positive patients, 246 (63.07%) were males and 144 (36.92%) were females. Among the total positive cases, there were 198 (50.76%) found positive for IgM and 98 (25.12%) were positive for IgG, while 56 (14.35%) patients were found positive for NS1 and 5 (1.28%) patients were positive for NS1+IgG. Similarly, 7 (1.79%), patients were found positive for NS1+IgG+IgM while 26 (6.66%) patients were positive for IgG+IgM (**Table 4**).

Table 4: Outcomes from THQ, Besham.

Anti-dengue Antibodies	Patients Number	Percentage%
IgM	198	50.76%
IgG	98	25.12%
NS1	56	14.35%
NS1+IgG	5	1.28%
NS1+IgG+IgM	7	1.79%
IgG+IgM	26	6.66%

Month wise result (August to December)

From August to December, total 1447 laboratory tests for determination of Dengue infection were conducted and the data was recorded on monthly basis to analyse month-wise prevalence of the Dengue outbreak in District Shangla (KP. Pakistan). In the starting month of August, total 270 individuals were screened in which 92 (34.07%) were positive i.e. 63 males (11.37%), and 29 females (5.23%) and 178 (65.92%) resulted as negative. Likewise, in the next month September,

overall, 382 tests were performed in which 203 (53.14%) individuals in which 147 (26.53%) males and 56 (10.10%) females found positive for dengue fever and 179 (46.85%) found negative. Total severity of dengue virus was (36.64%). In the month of October, a total of 397 blood samples from people were analyzed in which 186 (46.85%) was obtained positive in which 103 (18.59%) were male members and 83 (14.98%) were female members determined and 211 were negative. In the following month of November, 262 persons blood samples were tested in which 59 (22.51%) patients determined positive with dengue infection in which 36 (6.49%) were male patients and 23 (4.15%) were female diagnosed, while 203 found negative. Overall dengue outbreak of 13.17% was noticed. It the last month December, 136 tests were conducted in which 14 (10.29%) detected as positive which included 9(1.62%) males and 5 (0.90%) female, and 122 (89.70%) found as negative, as shown in **Figures 1 and 2**.

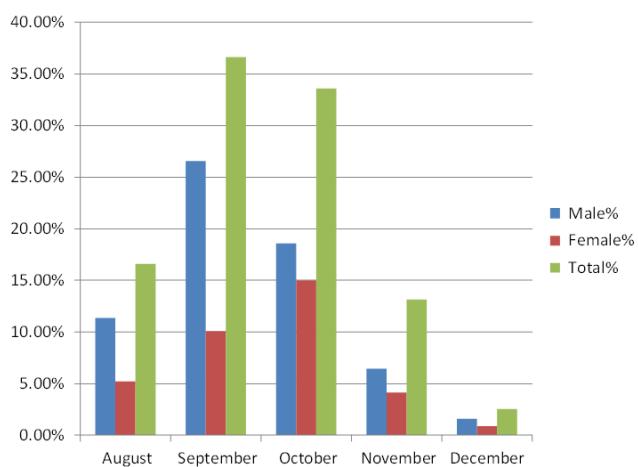


Figure 1: Month wise prevalence in male and female.

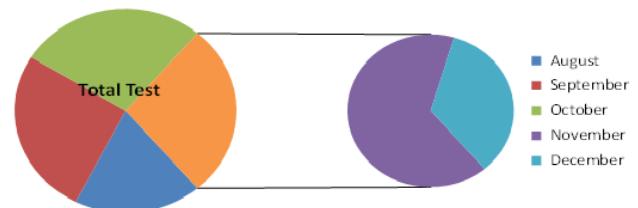


Figure 2: Month wise total conducted tests (positive and negative).

Clinical symptoms among the dengue infected population

During the course of studies, all the patients who visited both the above mentioned hospitals were monitored for clinical features of the disease which has been depicted in the graph below. Among them, fever was the most common

followed by spleenomegaly and abdominal pain. Other symptoms noticed that were common included vomiting, vomiting and internal bleeding in several organs such as nose and gums, while the skin rash was rarely found comparatively, as shown in **Figure 3**.

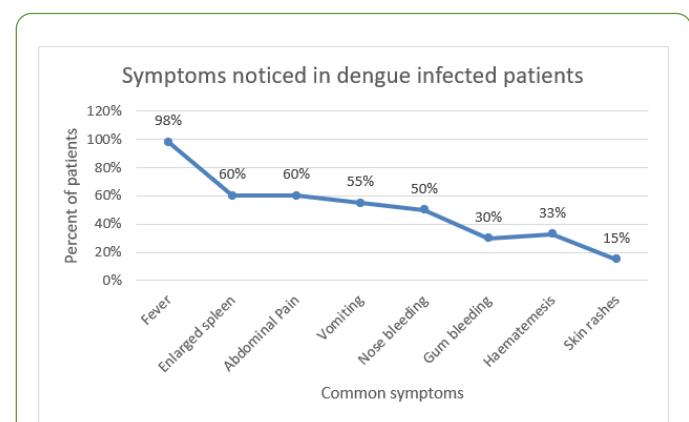


Figure 3: Symptoms observed in the DF positive individuals.

Discussion

It is believed that Dengue virus has entered Pakistan at Karachi seaport via tyres that carried eggs of the infected mosquitoes. So far, dengue virus has caused a number of epidemics in Pakistan [10]. Dengue infection was identified at Punjab, Pakistan in 1982 and 12 positive cases for dengue virus were reported out of 174 patients. Till 1994, no valid records were available about cases of dengue infection in Pakistan [11]. The most common symptom during the infection was the abdominal pain which was recorded in 51% of the patients. Along with this, 94% of the individuals were reported with splenomegaly. Almost many of these patients were anaemic. Their Hb level was <10 g% and platelets were between 50,000 cmm-100,000 cmm. Such results have also been noticed during the current research work. It was also observed that pain as the most frequent symptom in all the infected individuals followed by the enlargement of liver. Molecular diagnosis of this epidemic has previously been described by the presence of two dengue serotypes (DENV-2 and DENV-3). Here in our study, we detected that majority of the ill-people were suffering from bleeding from different parts of body such as nose, gums etc [12]. Data from the hospital about the biochemical evaluation showed that these individuals suffered with thrombocytopenia and leucopenia. From our study, it can be suggested to such patients to take fluids like juices frequently and using medicines which reduces or avoids the risks of thrombocytopenia and leucopenia. Furthermore, most positive cases among the patients were recorded in those people who travelled to an area where dengue fever was prevalent compared to the ones who have not visited such endemic sectors. A higher occurrence of both IgM and IgG was seen in urban areas than in rural areas. Along with the dengue fever, other complications persist which include fever, headache, joint pain, skin rash and fatigue. A scientist named Low et al. stated based on his studies at Singapore that

headache, fever, fatigue, joint pain, and skin rashes are statistically linked with dengue infection [9,13]. Similarly, we observed such symptoms in dengue patients (**Figure 3**).

The antibodies (IgM and IgG) were more commonly observed in the post-monsoon season (i.e. 68.33%) compared to that of the monsoon period (31.68%). A higher number of positive cases were found in males compared to female patients. While the study, it was quite evident that travel history plays vital role in the spread of dengue fever in geographical region. Based on this study, it is recommended to follow strict preventive measures for travels at both the national and international level. These measures can help prevent the spread of dengue infection from endemic region to the non-endemic region. The current study reports a higher rate of dengue infection in males compared to females (**Table 3**). The period of fever was monitored between 4-7 days, however many individuals showed remittent duration of fever.

DF is identified to be a disease of the urban sector and it is partially attributed to gradually more populated towns in rural areas as well [14]. Hospital records depicted that higher number of victims belonged to urban areas as compared to rural areas. Such results were also reported by Khan et al., [15]. In the present study, it came into observation that the post-monsoon season (August-September) (**Figure 1**), favoured highest dengue infections. It can be due to higher humidity after heavy rain fall, which provides suitable environment for mosquitoes breeding. Our results are similar to national report, and an international report regarding the dengue outbreaks [16,17].

Our sero-prevalence of dengue antibodies approximately (IgM=50% and IgG=25%) is higher than the ones based on epidemiological reports of study from Lahore. At Lahore, IgM incidence was 48.66% while that of IgG was 39.5%. Similar surveys conducted at Hyderabad, Sindh reported 16.47% IgM and 12.35% IgG. Also, in Khyber Pakhtunkhwa, an overall weighted prevalence of dengue-specific IgM and IgG antibodies was found to be 52.12% which included 31.86% positive for IgM and 20.26% positive for IgG in these patients [18,19].

In another study, a total of 341 acute cases were observed. 166 individuals (48.7%) were confirmed having raised antibodies against dengue IgM, while 27 (7.9%) patients fell in Gray zone and the rest 148 cases (43.4%) were found negative, because no specific antibody against the dengue virus was detected after 6 days of onset of symptoms. Our results are in accordance with the results of several previous studies relating to this topic [20-23].

Taking into consideration the present and previous studies, it is evident that dengue infections show cyclical variation with high epidemic and non-epidemic years. It mostly occurs in the form of massive outbreaks. However, dengue outbreaks vary with different seasons of the year. During a suitable season, high transmission of the disease is noticed and the factors like host characteristics, vector and the causative agent has a great influence in prevailing the outbreak.

Conclusion

There is a dire need to make efforts to preclude the dengue infection which is possible only if the policy makers and Khyber Pakhtunkhwa government take an initiative. Several strategies to curb the vectors using biological and/or environmental and chemical measures should be devised. Moreover, the importance of educating the people and awareness campaigns cannot be overlooked. Additionally, there is need to perform further research to investigate sero-prevalence on a large scale and determine the real magnitude of the problem. Furthermore, these results show a large earlier exposure of the compatriots of Shangla and Buner to dengue infection which further demands the critical analysis to identify the circulation patterns of the current dengue virus and characterize the associated serotypes and genotypes.

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Authors Contributions and Consent

Rehman AU contributed to the main idea, conceptualization and performing the research work. Haq IU contributed in the data collection and writing of manuscript. Asghar M, Afzidi GZ, Faisal S and Abdullah have contribution in formatting the manuscript draft and creating figures and tables. Ullah R, Zahir F, Abbas M, Ali F, Ullah H, Azam Q and Muhammad I have contribution in the statistical and critical analysis of the manuscript and also help in proofreading. All authors are agreeing to the publication of this article in Journal of Biomedical Sciences.

Conflict of Interest

We all declare no conflict of interest.

Ethical Approval

The study was performed with the approval of the Ethical Committee, Department of Genetics, Hazara University, Mansehra.

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