International Journal of Mosquito Research

Ihteshamul Haq
Graduate School of Biotechnology and oriental Medicine (Lab of Hangban Bio) Kyunghee University, Global Campus Suwon, South Korea

Fazli Zahir
Department of Allied Health Science Iqra National University Peshawar KP, Pakistan

Aziz ur Rehaman
Department of Biochemistry Rehman Medical College Phase 5 Hayatabad Peshawar KP, Pakistan

Noor Ullah
Institute of Basic Medical Sciences, Khyber Medical University Peshawar KP Pakistan

Jabir Khan
Department of Food Sciences and Nutritional Engineering, China Agriculture University Beijing, China

Siraj Ahmad
Department of Biotechnology and Genetic Engineering, Hazara University Mansehra KP, Pakistan

Waseem Sajjad
Department of Biotechnology and Genetic Engineering, Hazara University Mansehra KP, Pakistan

Abid Ur Rehman
Department of Biotechnology and Genetic Engineering, Hazara University Mansehra KP, Pakistan

Wajid Ali
Department of Biotechnology and Genetic Engineering, Hazara University Mansehra KP, Pakistan

Zesshan Qamar
Department of Biotechnology and Genetic Engineering, Hazara University Mansehra KP, Pakistan

Noman
Department of Biotechnology and Genetic Engineering, Hazara University Mansehra KP, Pakistan

Izaz Ali
Institute of Biotechnology and Genetic Engineering (IBGE) Agriculture University Peshawar KP, Pakistan

Younas Khan
Department of Biotechnology and Genetic Engineering, Hazara University Mansehra KP, Pakistan

Corresponding Author:
Jabir Khan
Department of Food Sciences and Nutritional Engineering, China Agriculture University Beijing, China

Evaluation of change in hematological parameters and epidemiological identification of dengue virus infection at district Peshawar, Khyber Pakhtunkhwa, Pakistan

Ihteshamul Haq, Fazli Zahir, Aziz ur Rehaman, Noor Ullah, Jabir Khan, Siraj Ahmad, Waseem Sajjad, Abid Ur Rehman, Wajid Ali, zesshan Qamar, Noman, Izaz Ali and Younas Khan

Abstract
Objective: The current study was performed to assess the Hematological aspects of dengue infected patients during 2018 at District Peshawar, KP Pakistan. It was done using serological tests to screen the infected patients for dengue. Patients who visited two main health centers (Khyber Teaching Hospital, Peshawar and Lady reading Hospital, Peshawar) of District Peshawar were taken into consideration for the study.
Methodology: During 2019, a total of 1537 visited these hospitals. Blood samples from the patients at pathology laboratory. Record of the patients was noted at the hospital. The data was taken from these registers 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.
Results: A total of 1537 blood samples were screened. Among them 449 were found positive for dengue fever whose acquired percentage was (37.27%), while 887 (59.60%) samples were determined with the negative outcome. With respects to patient's age groups, there were 269 patients found positive with Dengue infection ages of 16 to 334 years with a percentage of ((47.71%) which constitutes the majority of majority dengue infected patients. And individuals having age from one day to 14 years, there were 152 (28.27%) patients found with Dengue infection, which is the second highest number of individuals suffering dengue fever. Among the people in the ages of 36 to 60 years, 129 (23.23%) members showed a positive outcome for the infection. 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. During the month of August, only 88 (15.73%) patients were identified positive. In the month of September, 199 patients with (35.54%) percentage appeared with Dengue infection which is highest in the recorded study followed by 179 patients found positive in October with a percentage of 31.32%.In November. 204(34.32%) members were identified positive for by Dengue fever. And in the last month December, the total outbreak was (3.32%) with only 16 positive cases.
Conclusion: The prevalence of dengue outbreak in 2019 showed the increase rate in Dengue infection due to lack of awareness in individual and also the lack of proper health system. It emphasizes a strict need of Government attention, enactment and a strong policy constitution for the prevention of Dengue infection. 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 fact (What it mean?) and impacts of the dengue fever on the people, environment, intensions and traditions in different regions of the District.

Keywords: Dengue infection, hematology, epidemiology

Introduction

Dengue Fever is mosqutive-born infectious disease which is caused by Dengue virus (DENV) having single-stranded Positive-sense RNA that belong to the genus of Flavivirus and family Flaviviradae. The Prevelance ratio of Dengue infection is higher in the tropical region of the world. Dengue infection is tramsit to human through mosquito Aedes and having two form which is Dengue Fever (DF) and the Dengue Hemorrhagic Fever (DHF) which can cause the dengue shock syndrome [1]. Dengue Fever mainly spreads through mosquito vectors *Aedes aegypti* and *Aedes albopictus.* They belong to the order Deptera and culicidae family.

One of its four serotypes (DEN-1, DEN-2, DEN-3, and DEN-4) causes the disease. (Kraeger and Nathan, 2006) [2] Dengue is the chief aboviral disease to which humans are facing dominantly. According to their annual estimation, around 100 million Dengue Fever (DF) and several hundred thousand cases of hemorrhagic Fever (DHF) occurred [3]. Dengue is considered one of the most persistent illness in travelers from the areas where its enormous outbreak present [4] *Aedes aegthypti* is the most frequent vector of the Dengue outbreak has emerged from Africa and then expanded by the way of increase human travel, regarding trade all over the world, which is the prime contributing factor of the Dengue Fever proliferation [5]. The escalation of the Dengue virus in human occurs through the biting of *Aedes aegypti* which transmit the Dengue virus from infected person to non-infected one [6]. The serious problem of the Dengue outbreak is that it has been emerging upon re-infection with their one of the different serotypes which is the most alarming problem of their revival comes with high incidence of DHF, increase severity and huge frequency [7]. In Asian countries, many regions were encountered by Dengue severity mostly by Dengue Fever (DF) and Dengue hemorrhagic Fever (DHF) in 1998 [8].The Dengue outbreak existence has mostly occurred in the tropical regions. According to the annual estimation of the Dengue severity, there are 50100 million illnesses with Dengue Fever (DF), 250,000-500,000 with Dengue hemorrhagic fever (DHF) and 24,000 deaths has reported [9]. During 1987 in Thailand, regular outbreaks of the Dengue were outlined all over the state with intense severity in which more than 1000 deaths occurred. (Ministry of public health

Division of Epidemiology 1987). And further in 1998, there were 424 deaths occurred. (Ministry of Public Health, Department of communicable Disease control 200). In Thailand, Dengue severity was limited to the big cities but in aftermath, their prevalence frequency was found more in rural then urban zones [10]. In Southeast Asia, a freshly industrialized country, Singapore appeared with round about 3.6 million, Residents. Dengue revival was been estimated since 1986 [11]. In the year of 1997, the Dengue severity was found in Santiago de Cuba. The Cuban health Department in the late 1990s apprehended a danger of Dengue Endemics due to alteration in the epidemiological and economic bearings of the state [12]. In 1994, Pakistan confronted the first epidemic of Dengue hemorrhagic Fever (DHF) in Karachi, followed by years Dengue Fever outbreak in Baluchistan in the upcoming years [13]. Due to DENV-2, and DENV-3 serotypes revolution and consequent re-appearance, a major outbreak of dengue infection ravaged Pakistan in 2006. Later on, intense Dengue hemorrhagic Fever (DHF) was investigated using laboratory findings [14]. Recently in Pakistan, two immense and lethal outbreaks of Dengue Fever were reported which includes one in Lahore, the capital city of Punjab in 2011 and the other in Swat, KP in 2013 in which 20,000 people suffered from Dengue infection and nearly 4000 deaths occurred [15]. In 2013, Jehangir et al., found 320 patients positive for Dengue Fever during the span of 5 months in two Districts of KP, Pakistan i.e. District Shangla and District Bunir with 0% death rate and 100% Dengue Fever infection in the regions. [16].
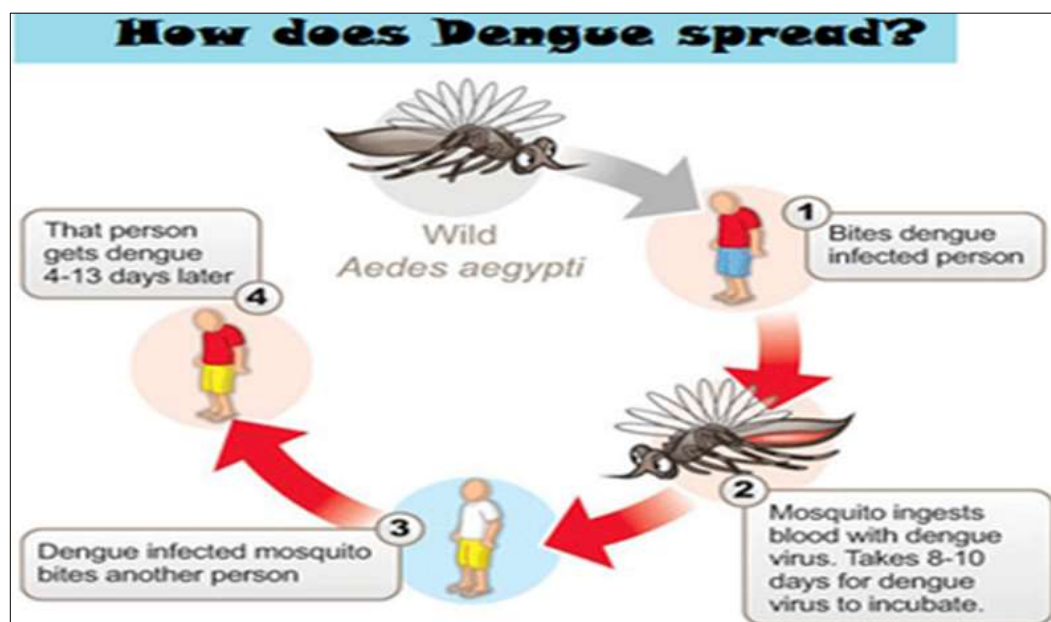

Fig 1: Show Dengue Infection Spread in Community.

Materials and methods
Study site
Peshawar district is situated in the province Khyber Pakhtunkhwa in Pakistan. The total area of the district is 1,257 square kilometers. (Wikipedia 2019) During 2019,

dengue severity was recorded in two main health centers of the district Peshawar Khyber epidemiological Teaching Hospital and Lady Reading Hospital Peshawar to assess the Hematological and based recent report of dengue fever.

Fig 2: Map of District Peshawar.

Data collection

Data of the patients was obtained from the registers of the above mentioned hospitals with the permission of the Medical superintendents. A separate sheet was used for taking extra details. Among the total visited patients, a total of 1537 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 the blood samples for the dengue fever identification in the visitors of the two selected health centers, 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 labeled 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 color 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 color line will appeared if relevant anti-Igg and anti-IGM antibodies of human immobilized in two test lines and make a complement through the test device. (Shu and Huang, 2004)

Dengue NS 1 Ag

In this test, firstly three drops (about 100μl) 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 color line appears within the result window which confirms a positive case. When two color 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 dropes. 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.
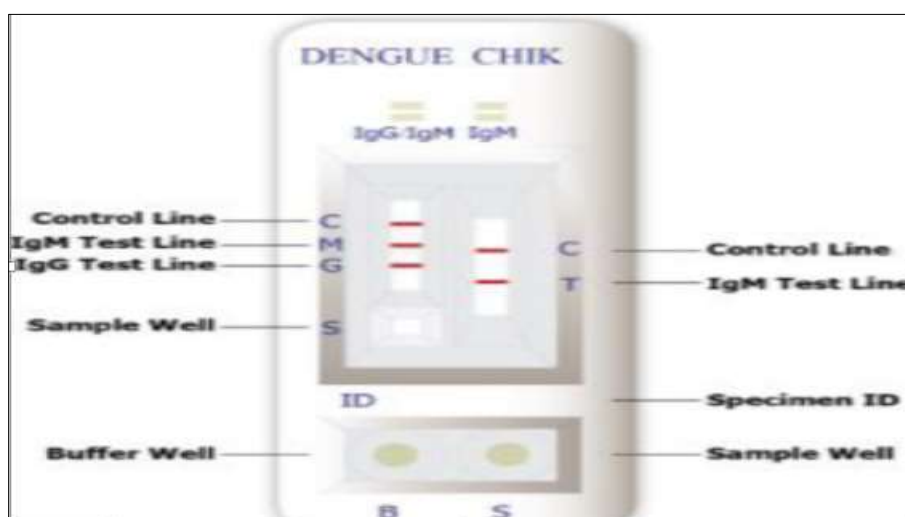
Fig 3: Test Kit for Igg and IGM Dengue Antibodies Detection.

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.

Fig 4: Show Positive and Negative result of Igg and IGM Antibodies in Dengue Patients.

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

Results
The current study was conducted from august to December to analyze the month-wise and age-wise Illness if dengue infection among the population of district Peshawar KP Pakistan. The diagnosis test of Dengue infection test were conducted in two main government hospital which is Lady reading Hospital (LRH) and Khyber teaching hospital Peshawar (KTH) Peshawar where total 1537 visitors patients screened and out of total 449 patients were found positive for Dengue Fever and 887 confirmed negative cases. According to Various diagnostic technique total 269 (47.71%) patients found positive with IGM (Anti-Dengue-Antibodies), 133 (23.24%) resulted positive with Igg (Anti-Dengue-Antibodies). And 89 (15.32%) resulted with NS1 (Anti-Dengue-Antibodies) and 6 (1.23%) positive cases obtained of NS1+Igg (Anti-Dengue-Antibodies).While 8(1.42%) patients with NS1+IgG+IgM, (Anti-Dengue-Antibodies) resulted. And, 35, patients with (6.32%) were obtained positive with Igg+IGM, (Anti-Dengue-Antibodies). Total male patients diagnosed were, 382 (69.41%) members whereas female patients were 173, (30.58%). In the total of 269 members diagnosed for IGM (Anti-Dengue-Antibodies), age wise estimation remained quite different. Out of 269 (47.71%) positive cases. patients from aged 1 to 15years is 67 (13.12%) 137 (26.04%) in the age group 15 to 35 years were positive, while in age category 35 to 60 years 65 (11.09%) patients showed positive result. In the result total recorded positive patients for Igg (Anti-Dengue-Antibodies) was 139(25.42%). Based on different age groups 42 (7.40%), 61 (10.83%), 36 (6.31%) cases were recorded for 0 to 15 years. 15 to 35 years and 35 to 60 years respectively. In the total 95(17.35%) positive patients with NS1 (Anti-Dengue-Antibodies), the number of patients for different age groups, i.e. 0 to 15years, 15 to 35 years and 35 to 60 years were 28 (5.12%), 44 (7.89%) and 23(4.21%) respectively. And the overall result for NS1+IgG (Anti-Dengue-Antibodies)) obtained, only 9 patients (1.31%) were there. In which the Age-wise estimation was, patients with Ages 0-15years, remained, 2 (0.19%) patient and for ages 15-35 years remained 7(2.04%) patients. With ages 35-60 years, remained (0%), as no

positive patients were found in this age group. While the total obtained result from Dengue patients' blood sample diagnosis for NS1+IgG+IgM, (Anti-Dengue-Antibodies) was (1.72%) and total positive patients were 11 in numbers, while the obtained results for age based categorization is 2 (0.36%), 9 (1.29%) and 0 for the above mentioned age groups. And the total result obtained for both Igg+IGM, (Anti-Dengue-

Antibodies) was (7.52%) with 41 positive patients. According to their Age-wise results patients with ages 0-15 years contained 12 positive patients (2.06%),while patients of age 15-35 years has 18 (3.12%) positive result. And patients with ages 35-60 years had 11 (1.91%) Iindividuals 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	188	81	269	47.71%
Igg	92	47	139	25.42%
NS1	64	31	95	17.35%
NS1+IgG	6	2	9	1.31%
NS1+Igg+IGM	9	2	11	1.72%
Igg+IGM	28	13	41	7.52%

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

Anti-dengue Antibodies.	0-15Years	15-35Years	35-60Years	Percentage%
IGM	67	137	65	47.71%
Igg	42	61	36	25.42%
NS1	28	44	23	17.35 %
NS1+Igg	2	7	0	1.31%
NS1+Igg+Igm	2	9	0	2.12%
IgG+IgM	12	18	11	7.52%

Khyber teaching hospital Peshawar (kth Peshawar) result
In the Pathology department of Khyber Teaching Hospital Peshawar, overall 493 tests were conducted in which 167(34.72%) were obtained positive for Dengue infection and 326 (63.72%) found with negative results. Among these, 63(36.53%) were female patients and 116(69.83%) were male patients. According to the overall diagnosed result of the patients with Anti-Dengue-Antibodies, there were 82 patients with percentage of (48.43%) found positive for IGM (Anti-Dengue-Antibodies) and 42(24.27%) patients found positive with Igg (Anti-Dengue-Antibodies) in their blood samples. While 39(22.53%) patient's blood samples were found positive for NS1 (Anti-Dengue-Antibodies). Hence for NSI+Igg+IGM (Anti-Dengue-Antibodies) there were 4(1.94%), patients' blood samples matched. While 12 (7.32%) patients' blood samples were obtained positive for Igg+IGM, (Anti-Dengue-Antibodies). (Table 3).

Table 3: Outcomes from Khyber Teaching Hospital Peshawar (KTH Peshawar)

Anti-Dengue Antibodies.	Patient Number.	Percentage%.
IGM	82	48.43%
Igg	42	24.27%
NS1	39	22.53%
NS1+Igg	4	1.94%
NS1+Igg+IGM	4	1.94%
Igg+IGM	12	7.32%

Lady Reading Hospital Peshawar (LRH, Peshawar)
In the Pathology department of Lady Reading Hospital Peshawar, total 993 blood samples of the visited patients to Hospital were examined for Dengue Fever Diagnosis, in which 407 (42.32%) were positive for Dengue Fever and 587 (60.43%) came out to be negative. Out of 407 positive patients which for Dengue infection, 251 (64.27%) were males and 149(37.83%) were females. Among the total positive cases, there were 204 (51.53%) found positive with

IGM (Anti-Dengue-Antibodies). and 103(26.32%) obtained positive with Igg (Anti-Dengue-Antibodies), while 63(16.36%), patients were found positive with NS1 (Anti-Dengue-Antibodies). And 8(2.32%) patients' blood samples matched with NS1+IgG (Anti-Dengue-Antibodies). Hence 9(2.57%), patients' blood samples found positive for NS1+Igg+IGM, (Anti-Dengue-Antibodies). While 29 (7.23%) patients' blood samples determined positive with IgG+IgM, (Anti-Dengue-Antibodies). (Table 4).

Table 4: Outcomes from Lady Reading Hospital Peshawar (LRH, Peshawar)

Anti-Dengue Antibodies.	Patient Number.	Percentage%.
IGM	204	51.53%
Igg	103	26.32%
NS1	63	16.36%
NS1+Igg	8	2.32%
NS1+Igg+IGM	11	2.93
Igg+IGM	29	7.32%

Month wise result (August to December)
In District Peshawar there are 1537 individual were screened for the detection of dengue infection from the month of august to December in district Peshawar KP Pakistan. In the first month of august there are 283 individual blood sample were analyze for the detection of dengue infection in which 99(35.91%)individual (66 males, 12.23%) and (32 females,6.12%) were determined positive with dengue infection and 184(67.12%) resulted as negative The total dengue outbreak was estimated (17.53%).Likewise in the next month September, total 392 tests were performed in which 209(54.23%) members in which 151(27.43%) males and 58(11.43%)females found positive with Dengue prevalence and 183(47.34%) found negative members. Total severity of dengue virus was (38.53%).While in the month of October, a total of 402 people's blood samples were analyzed in which 189(47.32%) was obtained positive for Dengue Fever in

which 104 (19.12%) were male members and 85(15.28%) were female members determined and 213 were negative. Dengue outbreak of 35.32% was detected. In the following month of November, totally 268 persons blood samples were tested in which 61(22.42%) patients determined positive with dengue infection in which 39(7.23%) were male patients and 22 (4.24%) were female diagnosed, while 207 found negative

members. Overall dengue outbreak of 15.27% was noticed. In the last month December, 141 tests were conducted in which 16 (11.12%) detected as positive for Dengue Fever which included 11(1.93%) males and 7(1.13%) females patients, and 125(89.70%) found as negative. The total outbreak was determined was 2.93%. As shown in Figures 5 and 6.
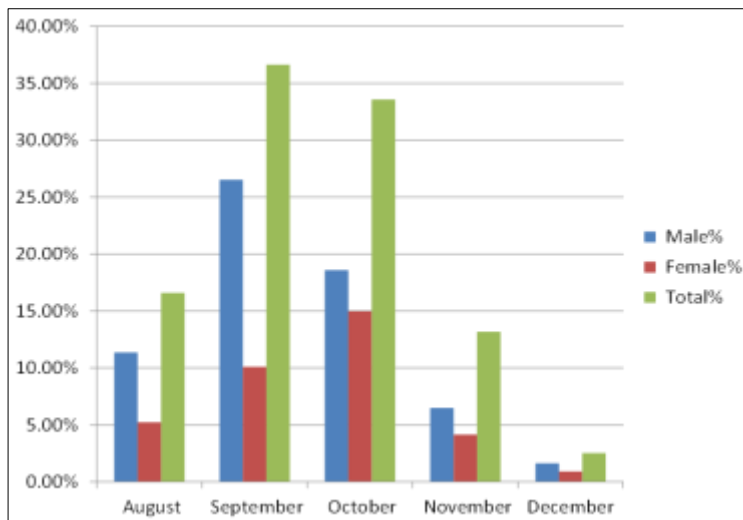

Fig 5: Month wise prevalence in male and female.

Fig 6: Month wise total conducted

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 was 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 7.
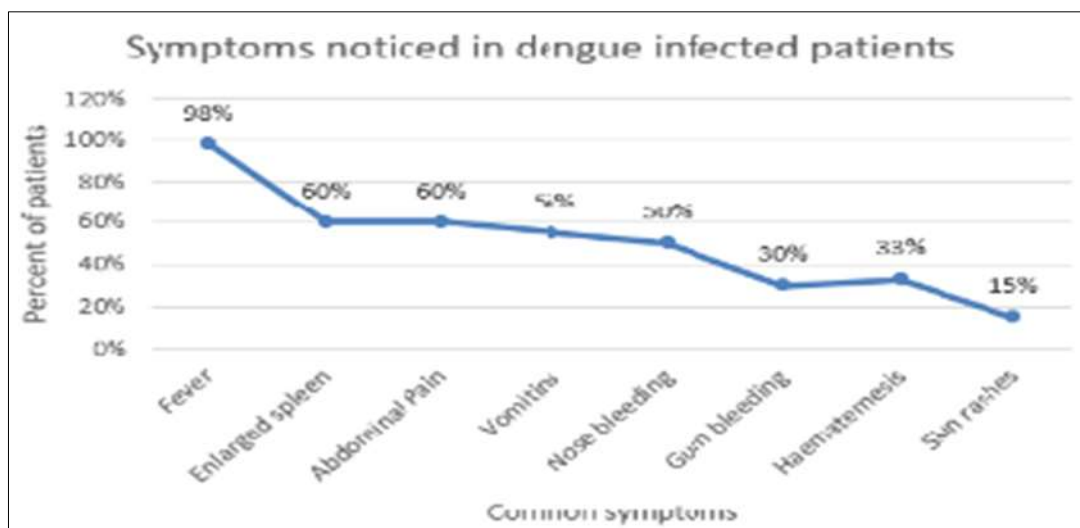

Fig 7: Symptoms Observed in the DF Positive Individual

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 [17]. Until 1994, there was no valid data available about dengue infection cases in Pakistan. In 1982, Dengue was identified in Pakistan, Punjab. Out of 174 patients, 12 were dengue virus positive [18]. 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 anemic. 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. [19]. 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 reduce or avoid 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 Patients [20]. Similarly, we observed such symptoms in dengue patients (Figure 7). 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 1). 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 attributted to gradually more populated towns in rural areas as well [21]. 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. [22]. In the present study, it came into Observation that the post-monsoon season (August-September) (Figure 5), favoured highest dengue Infection 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 [23]. 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 [24]. 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 Antibodies 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 [25]. Taking into consideration the present and previous studies, it is evident that dengue infection shows 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 characteristic 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.

Conflict of Interest

We all declare no conflict of interest.

References

1. Rehman A, Haq I, Asghar M, Afridi GZ, Faisal S. Sero-epidemiological Identification of Dengue Virus in Individuals at District Shangla, Khyber Pakhtunkhwa. Pakistan. J Biom dical Sci 2020,9(3).
2. Derouich M, Boutayeb A, Twizell E. A model of dengue fever. Biomed Eng. Online 2003;19(2(1)):4.
3. Ahmad I, Astari S, Tan M. Resistance of *Aedes aegypti (Diptera:Culicidea)* in 2006 to pyrethroid in Indonesia and its association with Oxidases and Estrases levels. Pak J Biol Sci 2007;10:3688-3692.
4. Ali S, Mahmood N, Afridi JZ, Jalil F, Haq IU, Ali I et al. HCV prevalence in the volunteer blood donors in

Peshawar, Khyber Pakhtunkhwa.

5. Lo pez-Ve, lez R, Tapia-Ruano C, Garcıa-Camacho A, Sanchez R. Dengue: a disease imported from the Indian subcontinent. Enferm Infecc Microbiol Clin 1994;12:182-6.

6. Anwar F, Tayyab M, Salman M, Abdullah Din M, Khan J, Haq I. Dengue outbreak 2018 in district Shangla KPK; clinical features and laboratory markers of dengue virus infection. *Future Virology* 2020;15(10),693-699.

7. Anwar F, Ahmad S, Haroon M, Haq IU, Khan HU, Khan J et al. Dengue virus epidemics: A recent report of 2017 from district Mardan, Khyber Pakhtunkhwa province, Pakistan. International Journal of Mosquito Research 2019;*6*(1):46-49.

8. Ahmad S, Anwar F, Ullah I, Alam M, Khan J, Abid-ur-Rehman FA et al. Epidemiological and clinical manifestation of dengue virus infection: A Recent Report from District Battagram Khyber Pakhtunkhwa, Pakistan 2018.

9. Shah IA, Anwar F, Haq IU, Anwar Y, Aizaz M, Ullah N. HBV Burden on Population, a Comparative Study between Two Districts Mardan and Charsadda of KPK, Pakistan. International Journal of Contemporary Research and Review 2018;9(09):20269-20274.

10. OOI EE, Hart TJ, Tan HC, Chan SH. Dengue seroepidemiology in Singapore. Lancet 2001;357:685-686.

11. Kourı G, Guzman MG, Valdes L et al. Reemergence of dengue in Cuba: A 1997 epidemic in Santiago de Cuba. Emerging Infectious Diseases 1998;4:89-92.

12. Paul RE, Patel AY, Mirza S, Fisher-Hoch SP, Luby SP. Expansion of epidemic dengue viral infections to Pakistan. INT J Infect Dis 1998;2:197-201.

13. Khan E, Siddiqui J, Shakoor S, Mehraj V, Jamil B, *et al.* Dengue outbreak in Karachi, Pakistan, 2006: experience at a tertiary care center. Trans R Soc Trop Med Hyg 2007;101(11):1114-1119.

14. Haq I, Ullah R, Din M, Ahmad S, Anwar F, Ali M et al. Unrecognized HIV infection in asymptomatic volunteer blood donors at district Peshawar, Khyber Pakhtunkhwa, Pakistan. New Microbes and New Infections 2020,100685.

15. World Health Organization. Dengue haemorrhagic fever: diagnosis, treatment, prevention and control, 2nd edn. Geneva: WHO1997.

16. Murray NE, Quam MB, Wilder Smith A. Epidemiology of dengue: Past, present and future prospects. Clin Epidemiol 2013;5:299-309.

17. ul Haq I, Khan M, Rehman Z, Anwar F, Ullah H, Ullah N. HBV prevalence in the volunteer blood donors in Peshawar, Khyber Pakhtunkhwa Pakistan Int. J Biosci 2018;13(5):50-54,

18. Mahmood K, Jameel T, Aslam FH, Tahir M. Incidence of dengue haemorrhagic fever in local population of Lahore, Pakistan. Biomedica 2009;25: 93-6.

19. Khaskheli A, Qazi N, Mahadev H, Ujan I. pattern of dengue virus infection cases admitted in teritarry care hospital at Hyderabad, Sindh. J Liaq Univ Med Health Sci 2010;9:142-144.

20. Sajid A, Ikram A, Ahmed M. Dengue fever outbreak 2011: Clinical Profile of children Presenting at Madina teaching hospital Faisalabad. J Uni Med Dent Coll 2012;3:42-47.

21. Haq I, Muhammad A, Fazli Zahir MK, Anwar F, Akhtar MS, Ullah F. Serological and Epidemiology study of Helicobacter pylori infection among Dyspeptic patients in District Peshawar Pakistan. Adv. Biores 2020:11(3):81-85.

22. Akram DS, Igarashi A, Takasu T. Dengue virus infection among children with undiffrenciated fever in Karachi. Ind J Pediatr 1998;65:735-740.

23. Abdullah Ali S, Salman M, Din M, Khan K et al. Dengue outbreaks in Khyber Pakhtunkhwa (KPK), Pakistan in 2017: An Integrated Disease Surveillance and Response System (IDSRS)-Based Report. Pol J Microbiol 2019;68:115-119.

24. Humayoun MA, Waseem T, Jawa AA, Hashmi MS, Akram J. Multiple dengue serotypes and high frequency of dengue haemorrhagic fever at two łĞrϴĂrLJ care hospitals in Lahore during the 2008 dengue virus outbreak in Punjab, Pakistan. Int J Infect Dis 2010;14:54-59.

25. Chareonsook O, Foy HM, Teeraratkul A, Silarug N. Changing epidemiology of dengue hemorrhagic fever in Thailand. Epidemiology and Infection 1998;122:161-166.

26. Gubler DJ. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. Trends Microbiol 2002;10:100-3.