Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2021 18(4): 2999-3014. **OPEN ACCESS**

Detection of Dengue virus in suspected patients of 2013-2014 Dengue outbreak in Swat, khyber pakhtunkhwa, Pakistan

Fawad Ali[1], Farhad Ali[1], Muhammad Shakeel[1], Muhammad Junaid Yousaf [2], Salma Noreen[3], Kaleem Ahmad[4], Rakhshinda Sadiq[5], Nadia Sharif [5], Saeeda Saeeda[6] and Asma Shah[5],

[1]Institute of Biotechnology and Microbiology, Bacha Khan University, Charsadda 4460, KPK, **Pakistan**
[2] Department of Botany Abdul Wali Khan University Mardan KPK, **Pakistan**
[3]Department of Botany, Women University Mardan KPK, **Pakistan**
[4]Department of Agronomy, University of Agriculture, Peshawar, (Amir Muhammad Khan Campus Mardan) **Pakistan**
[5]Department of Biotechnology Women University Mardan KPK, **Pakistan**
[6]Department of Botany Hazara University Dhodial Mansehra KPK, **Pakistan**

***Correspondence:** fawadansi@gmail.com **Received 02-08-2021, Revised: 17-10-2021, Accepted: 18-10-2021 e-Published: 05-12-2021**

The study was conducted on identification of dengue virus in suspected patients of 2013-14 dengue outbreak in District Swat and to conduct the population base surveillance for determining the proportion of dengue virus infection, dengue incident and disease burden. The total number of patients observed were 8927 out of which 6074 (68.1%) were male and 2853 (31.9%) were female. The male population were observed to be more affected by the infection than females, while the infection rate among male and female population below the age of 15 was lowest. The total number of deaths reported due to dengue infection were 36. The infection rate was much higher among the age group of 15-30 years. The characteristics symptom of dengue infection was recorded as fever (100%), vomiting (70.18%), abdominal pain (50.16%), and splenomegaly (34.16%). In majority of the patient's platelet count ranged between 100,000-150,000/mm, fever duration in most of the patient was 7-15 days. The fever duration also varied among the dengue infected individuals with 17.2% patients having continuous fever up to many days, 55.1% having intermittent and 26.6% patients having remittent fever. For the conformation of DHF (dengue haemorrhagic fever) the tourniquet test was done and by the symptoms of patients' condition petechiae was observed in 18.66% and bleeding of mucosa in 2.56%.

Keywords: Dengue ,District swat ,Infection.

INTRODUCTION

Dengue virus is a spherical single stranded virus that belongs to viral family, Flaviviridae and genus Flavivirus that affects mammalian and vector cells. Four various serotypes of dengue virus are acknowledged causing the disease (DENV-1, DENV-2, DENV-3 and DENV-4) and *Aedes aegypti* mosquito is the basic transmitter. Infection caused by one serotype provides a lasting immunity against that particular serotype, whoever subsequent infection by a different serotype increases the risk factor of developing fatal dengue (Centres for Disease Control and Prevention (CDC), 2000). Dengue fever is a fatal disease, having the symptom of severe flue, sickness, headache, pain behind eye, vomiting

and rashes on the body, and in severe cases it causes plasma leakage, watery accumulation, fatal bleeding or an organ impairment (Abbas et al.2014)

This fever is considered as bone breaker fever in infected person. Its symptoms vary from age to age. The effected person is suspected when high fever accompanied by server headache, pain behind eye, vomiting and red spot appear on the body. Vomiting, swollen glands, muscle and joint pains, increase breathing rate, bleeding gums, blood release during vomit, fatigue last 2 to 3 days. 24 -48 hours are critical and might be fatal for the patient. Medical care if not timely provided increase the complexity and risk (2).

Dengue virus is an arthropod-born viral infection around the globe, 975 million out of 2.5 billion people were at risk of dengue infection from the large and the small regions of the tropical and sub-tropical area.An exhaustive increase occurred in recent years, especially in America Western Pacific and South-Eastern Asia was observed for the last 5 years. Dengue virus infection depends on the serotype and an increase of 30 times more infection rate is observed presently in both urban and rural areas(Khan et al.2008) Dengue virus is a spherical single stranded virus that belongs to viral family, Flaviviridae and genus Flavivirus that affects mammalian and vector cells. Four various serotypes of dengue virus are acknowledged causing the disease (DENV-1, DENV-2, DENV-3 and DENV-4) and *Aedes aegypti* mosquito is the basic transmitter. Infection caused by one serotype provides a lasting immunity against that particular serotype, whoever subsequent infection by a different serotype increases the risk factor of developing fatal dengue (Centres for Disease Control and Prevention (CDC), 2000). Dengue fever is a fatal disease, having the symptom of severe flue, sickness, headache, pain behind eye, vomiting and rashes on the body, and in severe cases it causes plasma leakage, watery accumulation, fatal bleeding or an organ impairment (4).

According to the survey of World Health Organization (WHO) 50 million dengue infections are reported globally every year. Now it is declared that it is an endemic in South Asia. Dengue fever covered a very large area of Pakistan. The spread of dengue infection may be contained in the sub-tropical areas due to a fine environment (Butt et al. 2011).This fever is considered as bone breaker fever in infected person. Its symptoms vary from age to age. The effected person is suspected when high fever accompanied by server headache, pain behind eye, vomiting and red spot appear on the body. Vomiting, swollen glands, muscle and joint pains, increase breathing rate, bleeding gums, blood release during vomit, fatigue last 2 to 3 days. 24 -48 hours are critical and might be fatal for the patient. Medical care if not timely provided increase the complexity and risk (Chong et al.1994).

et al.2004 studies show that 351 dengue McAvin patients consisting of 68 primary and 283 patients having secondary infection in Binh Thuan province of Vietnam. In 25% cases dengue infection was confirmed through RT-PCR. Among the RT-PCR confirmed cases 32 were infected with DENV-1, 16 with DENV-2, 1 with DENV-3 and 37 with DENV-4. The data collected during their study indicated that the major serotype prevailing among the infected patients in 2001-02 was DENV-4 and in 2003-06 was DENV-1 and DENV-2. It was also observed during the study that children were mostly infected with Primary dengue. The study also identified higher variaemia level among the primary dengue infected patients as compared to secondary cases. Clinical symptoms of dengue infection were more visible in adults in comparison to children however no visible differences were recorded among primary and secondary dengue infection as well as for the different serotypes.

Thai et al.2010 reported that Dengue viral disease spread by the bit of mosquitoes. For identification of a dengue serotype responsible for infection requires the use of RT- PCR. Virus infected mosquitoes; both male and female can be detected on the NSI strip which can marked as infected in less than 3 minutes. The study focussed on the detection of dengue virus in mosquitoes and real time monitoring and control responses.

Muller et al. 2012 investigated that Dengue fever is a mosquito born disease. The study conducted used NS1 Ag Strip for the detection of dengue virus (DENV) antigen in the wild population of *Aedes Aegypti* mosquito. The accuracy of the results obtained in the research was comparable to the reverse PCR based results. Since, currently no known cure is available for the dengue infection; hence detection/ surveillance of dengue vector can serve as an important benchmark for the control and spread of dengue infection. This kit can generate results in an hour with minimal technical training required on part of the investigators.

Paul et al.1998 reported the outbreak of dengue in Pakistan in 1992 where 4500 dengue cases were identified. A large number of people were infected in Kashmir in 2006 but a substantial number of cases of dengue infection remained unreported. In 2010 a total of 21,204 cases of dengue infection were reported in Punjab that alarmed the government. In Punjab dengue infection in 2011 resulted in 350 reported deaths out of 16000 total dengue infections reported, while in Lahore alone 300 fatalities were recorded out of 14000 cases of reported infection in private healthcare hospitals.

Akram et al.1998 reported that Dengue fever occur in tropical and sub-tropical region through infection by one or more of four viral serotype DENV-1, DENV-2, DENV-3 and DENV-4. The predominant vector identified for the spread of dengue infection was *Aedes aegypti*.

MATERIALS AND METHODS

Immuno chromatographic method for detection of NS1 antigen

The SD BIOLOINE Dengue rapid kit was used for confirmation of dengue infection among the clinically diagnosed dengue suspected patients. This is one step assay designed to detect dengue virus NS1 antigen in human serum, plasma, or whole blood.

Procedure

With a disposable dropper 3 drops of serum were added into sample well. As the test begins to work, purple colour moves across the result window. Test results were interpreted at 15- 20 min.

Interpretation

The presence of one colour line (C band) within the result window indicates negative result. Whereas presence of two colour lines (T band and C band) within the result window indicates positive result. If the colour line was not visible within the result window, then the test was considered as invalid.

Detection of Dengue infection breeding sites Method and Material of affected area.

For identification of Dengue vector site a survey was conducted in collaboration with District Health officer Gulkada District Swat. During the Survey, dengue vector Breeding sites, i.e. fresh water standing sites were identified and samples were collected in plastic bottles which were clearly labelled. For identification of breeding site 30-40 ml of water sample were collected from each site. These samples were later observed under microscope for determination of vector larvae presence.

Affected Area.

For identification of affected area by dengue infection, the suspected patient's data were recorded on a questioner containing patient information along with their locality.

Prevailing weather condition.

The data regarding the prevailing weather condition i.e average temperature during the dengue virus infection pertaining to the 2013-14 dengue outbreak were collected from Meteorological office of district Swat.

RESULTS

Different regions of district Swat were brought under considerations which were adversely effected by dengue virus including Mingora, Kanju, Said Sharif, Patehpu, Watkay, Barikot & Matta while some patients belong to rural areas of Matiltan, Charbagh, Khawzakhela, Manglawar & Salampur of district Swat.

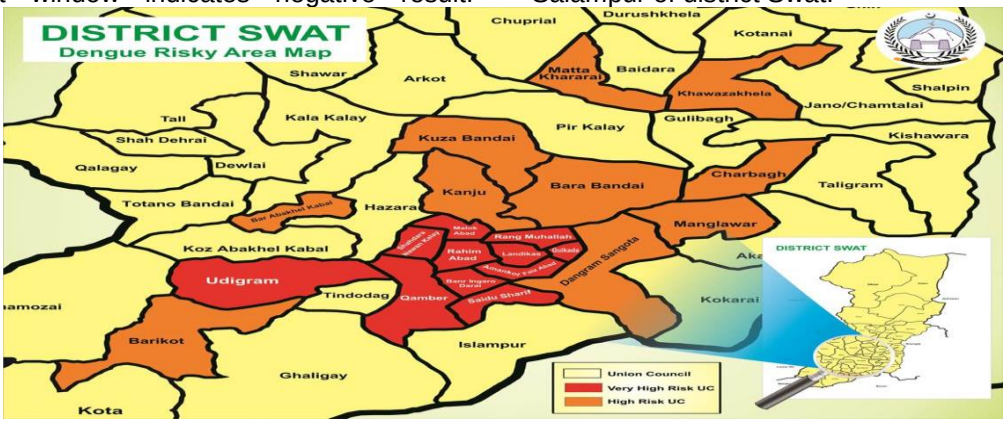

Figure 1: Map of District Swat

The research was carried out with the collaboration of Saidu Teaching Hospital and Shifa Medical Centre. During our research five ml blood sample was collected from each patient in different hospitals along with a proforma containing the information of respective patients. The serum from the blood samples was isolated in Eppendorf tubes through centrifuge machine and preserved at -80 ^{0}C.

Table 1: Very High Risk and High Risk Area of District Swat

District Swat Tehsil Babozai (Mingora city is divided In the following high risk union councils)	**Other high risk union councils of District Swat**
1)Rahimabad *2)Banr Engaro Dherai* *3)Gulkada* *4)Qambar* *5)Amankot Faiz abad* *6)Landi Kass* *7)Odigram* *8)Shahdara* *9)Saidu Sharif* *10)Rang Mhalla* *11)Malook abad*	*1.Matta Kharirai* *2.Khwazakhela* *3.Bara Bandai* *4.Koza Banda* *5.Kanju* *6. Charbagh* *7.Manglawar* *8.Dangram sangota* *9. Bar abakhel* *10. Barikot*

Dengue fever situation in district Swat, Khyber Pakhtunkhwa (August 07 – October 31, 2013):

We investigated 8,927 cases of Dengue fever & DHF patients out of which 6074 (i.e. 68.1%) were male and 2853 (i.e. 31.9%) females (Figure No 4). Most of the patient belonged to the urban area and were among the age group of 15-30 years. Figure No 5 shows the age and sex wise distribution ratio of dengue infected patients.

Total cases of dengue infection reported= **8,927** (males=6074, females=2853)

Total deaths reported to be caused by dengue infection= **36**

Dengue infection among population

Among the highly-infected Union Councils of District Swat the rate of infection was higher in urban areas as compared to rural. In Saidu Sharif infection was observed at 35%, Mingora city 27%, Matta 12%, Kanju 9%, Patehpur 04%, Charbagh 7% and Khwazakhela 6%

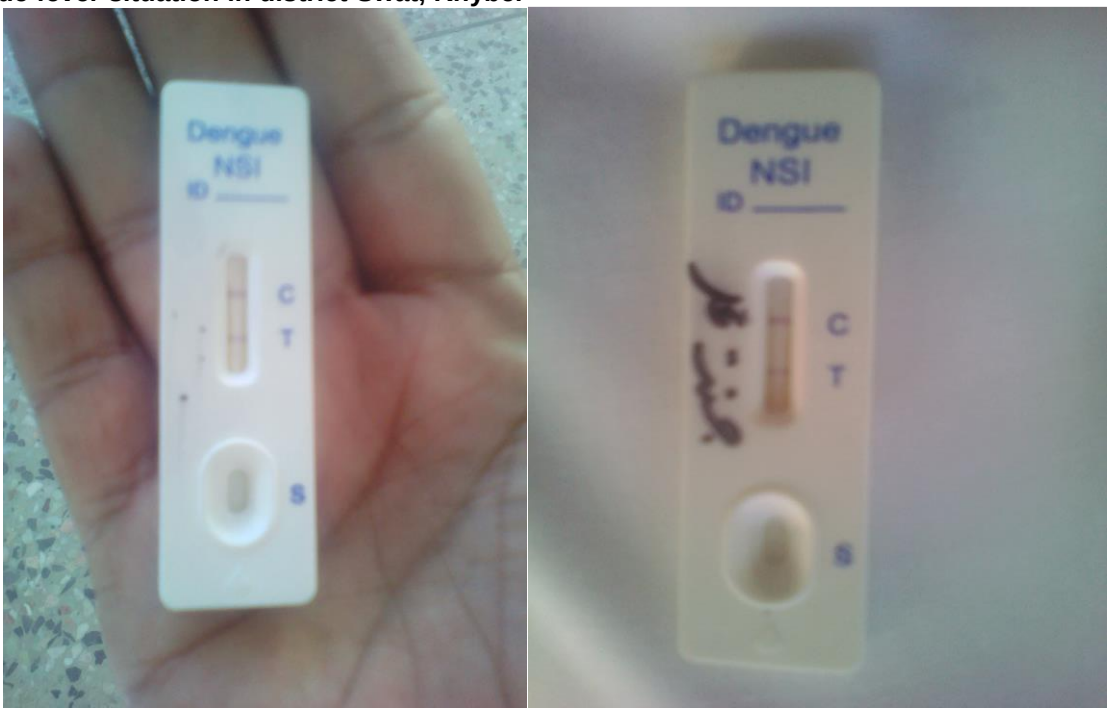

Figure 2: Dengue Positive strip

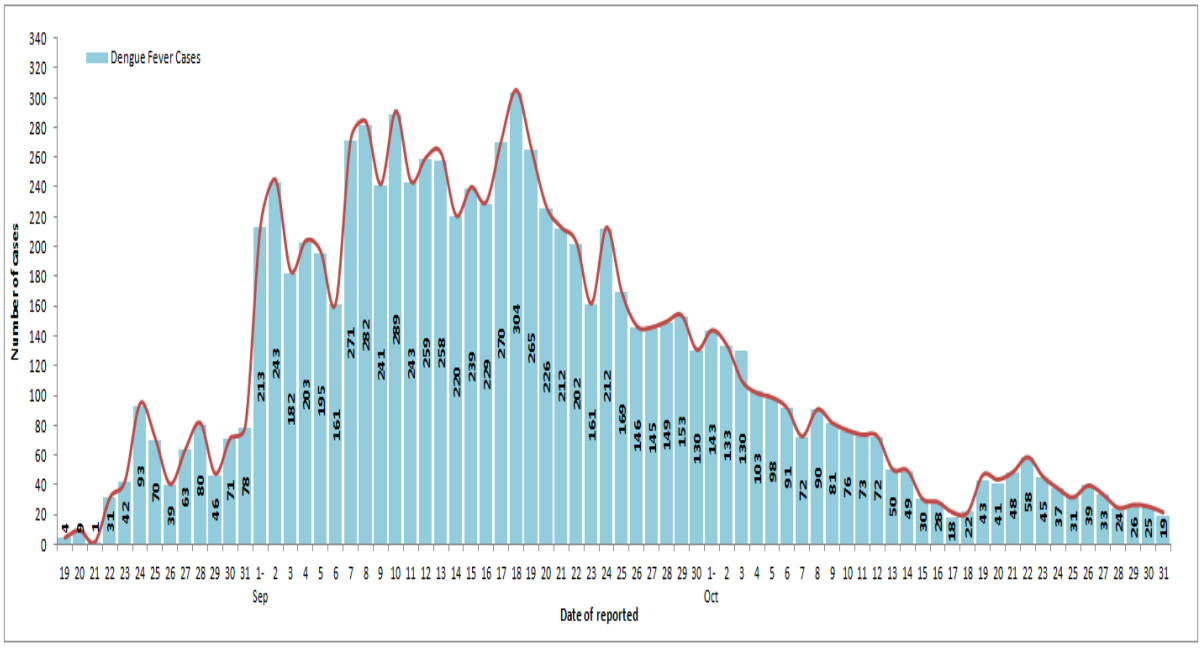

Figure 3: Daily dengue fever cases confirmed

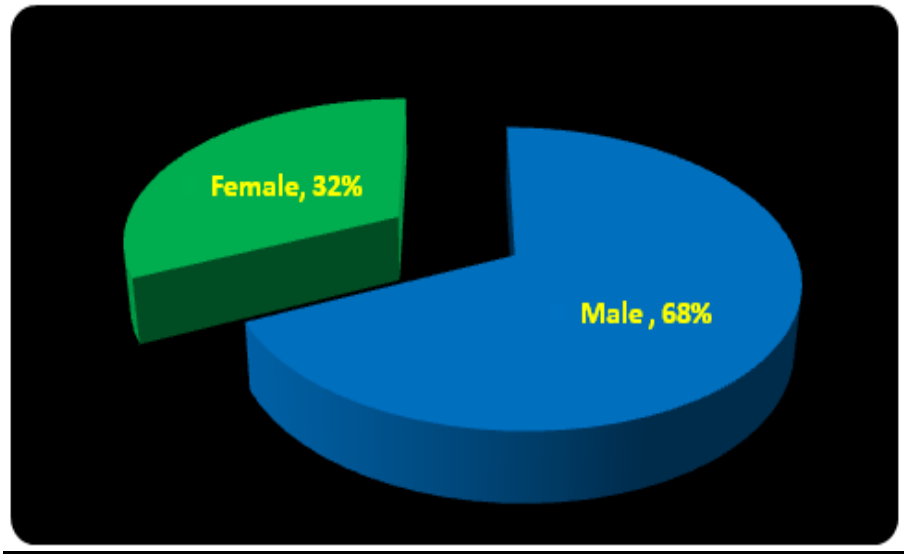

Figure 4: Gender wise distribution of dengue fever:

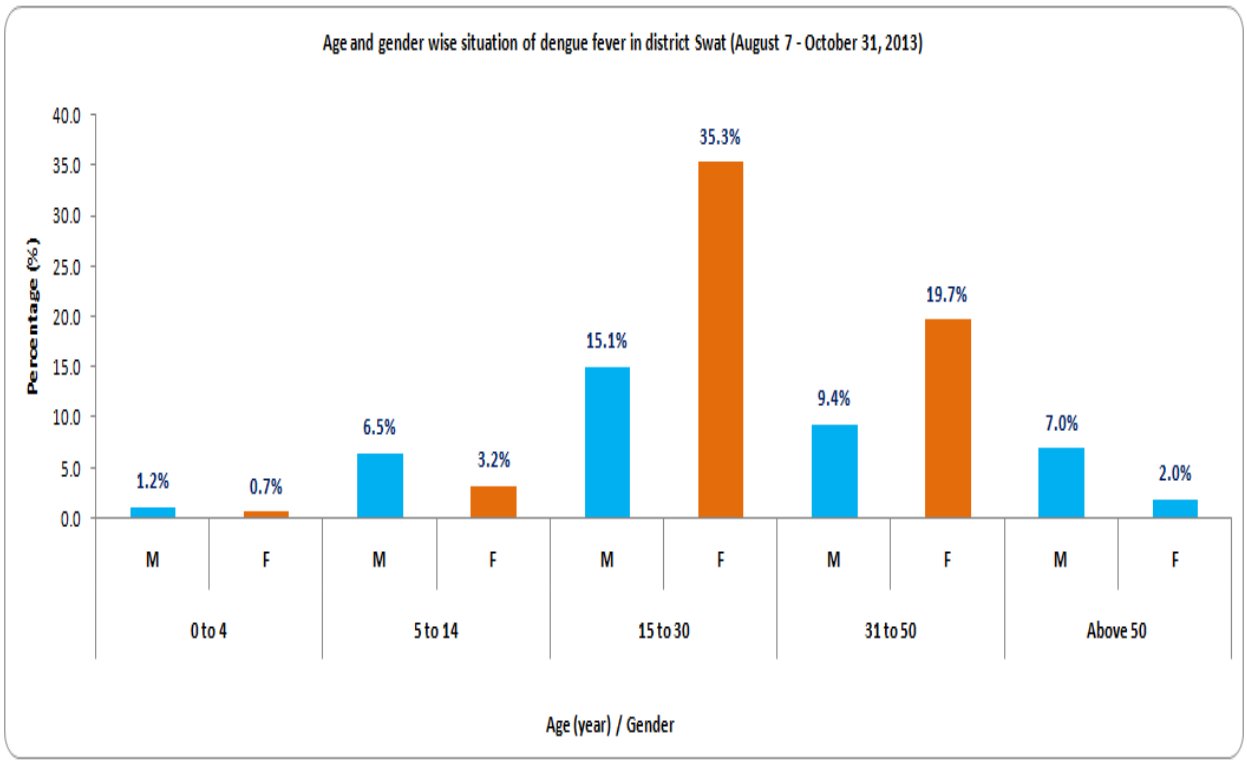

Figure 5: Age and gender proportion segregation of dengue fever cases.

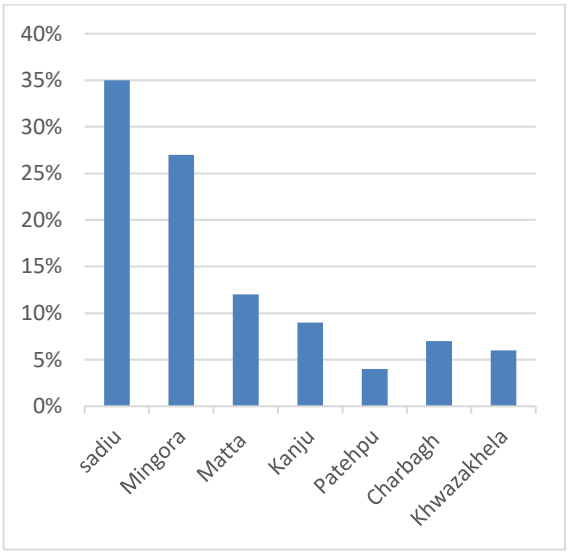

Figure 6: showing infection rate among different area of District swat

Clinical findings

The most common symptom among the infected patient reported was fever (100%), vomiting (70.18%), abdominal pain (50.16%), while in some patients skin rashes (Petechiae) (18.66%), gum bleeding (2.65%), & Splenomegaly (34.16%) was also observed (Table No 2 and Figure No 7).

Table 2: Patients presenting signs and symptoms of dengue fever (n=8927)

Symptom	Percentage
Fever	100%
Vomiting	70.18%
Abdominal pain	50.16%
Skin rashes (Petechiae)	18.66%
Gum bleeding	2.65%
Splenomegaly	34.16%

Table No 2: Patients presenting signs & symptoms of dengue fever (n=8927)

During the current research among the patients identified with dengue fever and DHF, 1540(17.2%) patients had continuous condition of fever up to many days, 4920 (55.1%) was presenting intermittent and 2470 (26.6%) patients with remittent fever.

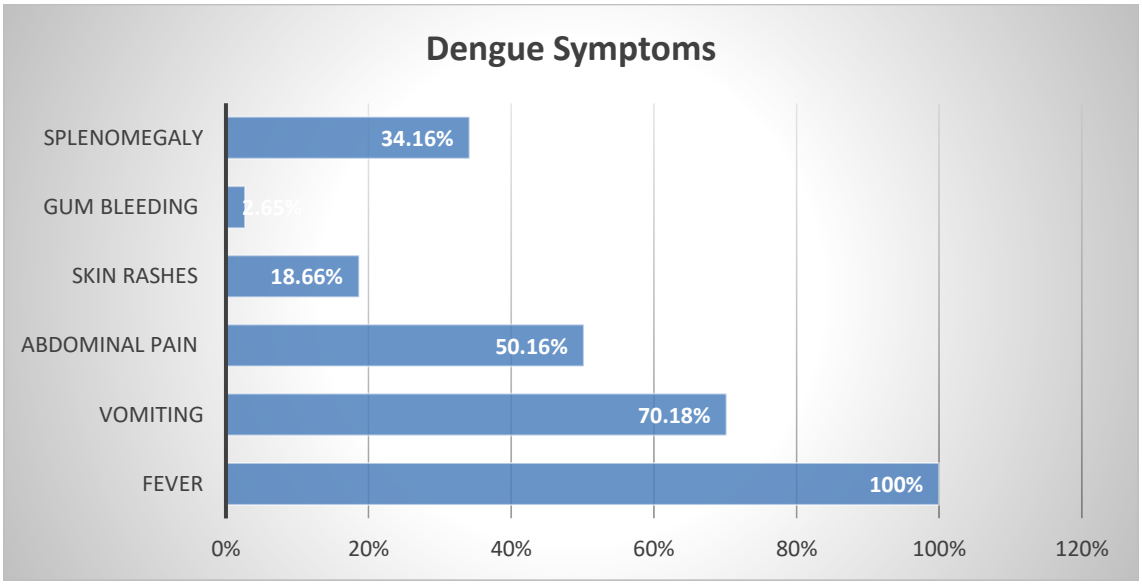

Figure 7: Patients presenting signs & symptoms of dengue fever (n=8927)

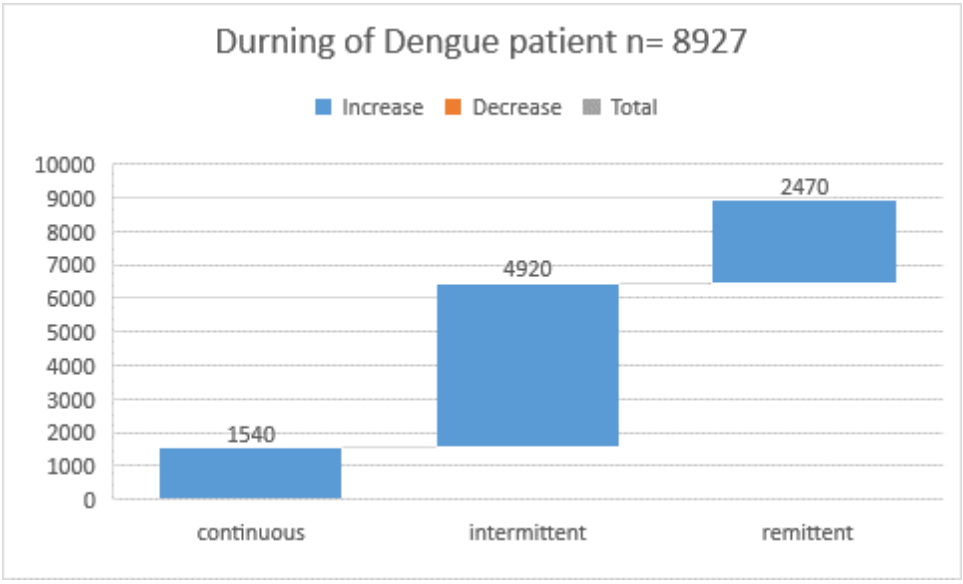

Figure 8: show condition of Fever among the patients

For platelets count 500 dengue patients' blood samples were tested. The platelets count in 96 (15.16%) patients were less than 50,000/cmm, in 102 (28.65%) patients it ranges from 50,000-100,000/cmm while in 302 (56.18%) patients the platelets count was from 100,000 to 150,000/cmm.

The rate of infection was low in June (14.8%) due to relatively low temperature of an average 50.2 ^{0}F and gradually increased in start of the rainy season in July (17.1%) with an average temperature of 55.4^{0}F and the rate of infection was much higher (27.1%) in August with an average temperature of 48.2 ^{0}F. In September, the rate is of infection was recorded at (22.7%) due to decrease in temperature (47.3^{0}F) and rainfall, it might be due to the well-established breeding of *Aedes* vectors for dengue virus) in raining months

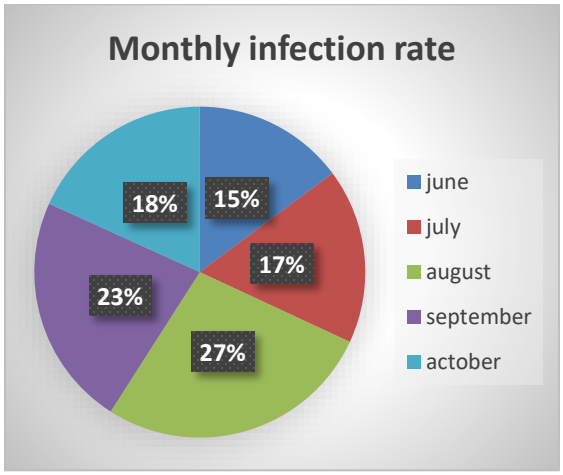

Figure 9: Month wise distribution of dengue infection (n=8927)

Table 3: Month wise distribution of dengue infection (n=8927)

June	July	August	September	October
1324 (14.8) %	1527 (17.1) %	2424 (27.1) %	2027 (22.7) %	1624 (18.2) %
50.2 ^{0}F	55.4 ^{0}F	48.2 ^{0}F	47.3 ^{0}F	46.4 ^{0}F

In the current research, we have calculated different outdoor breeding sites in District Swat. The data recorded is presented in table No.4 and Fig No 11, The highest number of breeding sites were identified in water tanks and drums 5736 followed by tyres 4613 and plant pots 3442 while in open drain gutters the lowest number of breeding sites i.e. 72 were identified followed by water pots for tyre puncture 23.

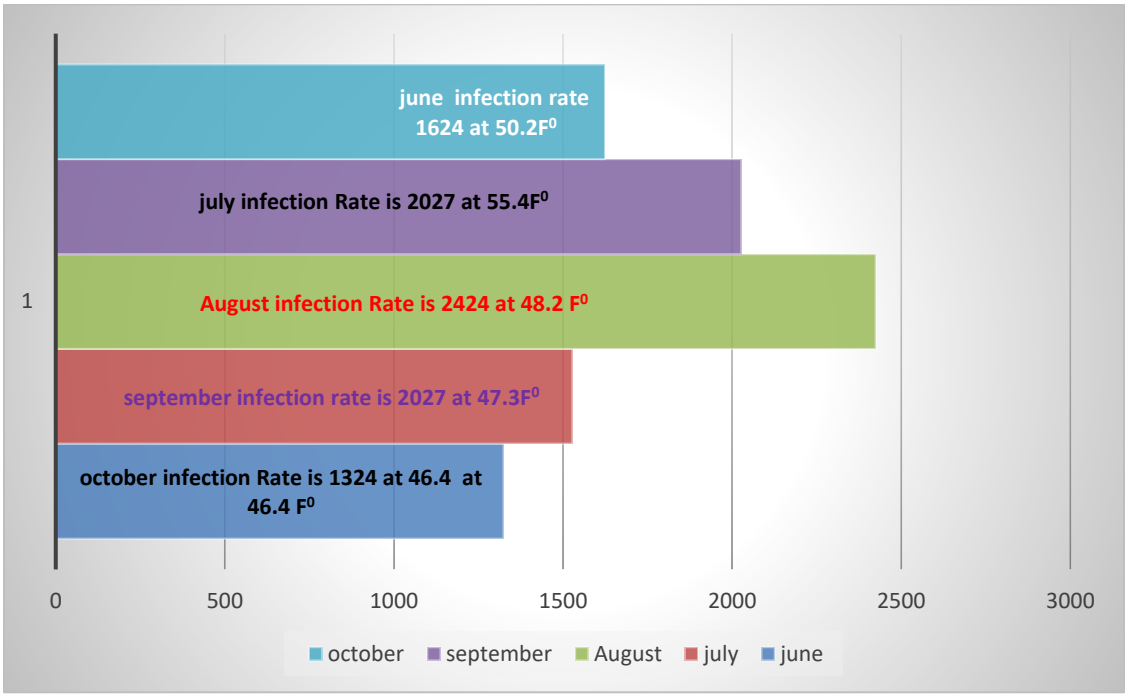

Figure 10: Infection rate distribution in different months

Table 4: % age of potential breeding sites in Outdoor survey

S.No	Name Specific Site	Number of Sites	% age
1	water tanks and drum	5736	24
2	Tyres	4613	19.3
3	Plant pots	3442	14.4
4	Standing water	2390	10
5	Waste material	1434	6
6	Other (Block drains, drums, Vehicle discarded parts)	1195	5
7	Leaking water taps	1673	7
8	Bird water pots	478	2
9	Air cooler	478	2
10	Air condition water	239	1
11	Road side Hedgerow	717	3
12	Tree holes	239	1
13	Open drain/ gutter	72	0.3
14	Park bushes	478	2
15	Water pot for tyre puncture	236	1
16	Construction material	478	2
	Total	23898	100

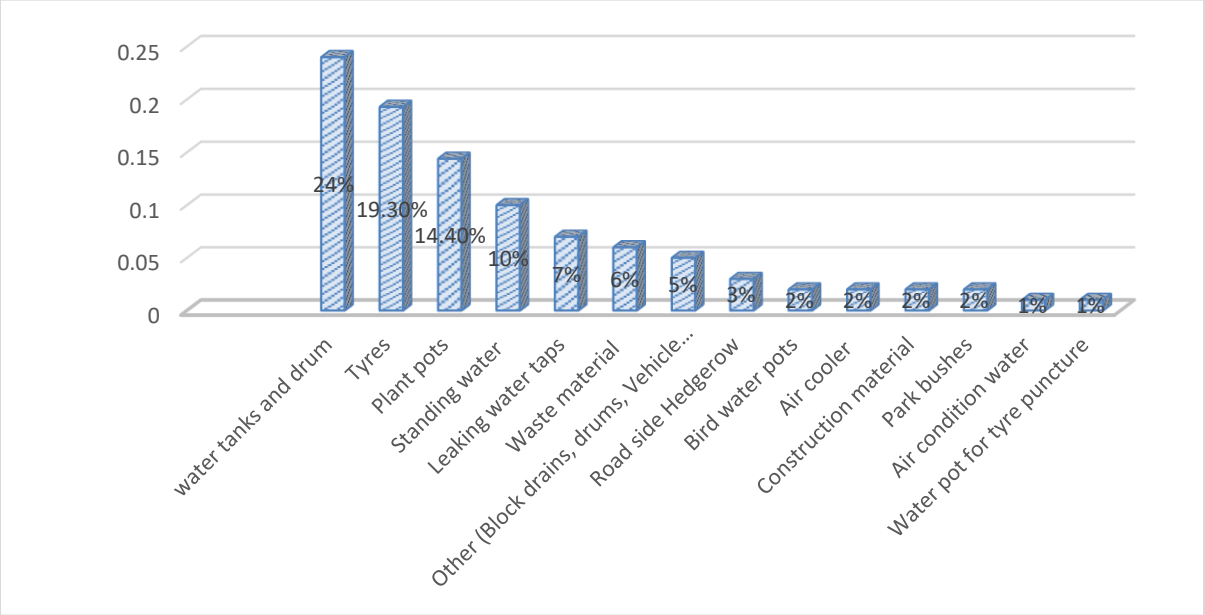

Figure 11: % age of potential breeding sites in Outdoor survey

In the current research we have calculated total number of different indoor potential breeding sites in District Swat including Standing water in the houses, leaking Water Taps, Construction waste, Cover of Gutter, Old Tyres in the houses, Mini Plant Pots, Discarded Material, Animal water utensils, Air cooler Excretion water, Store water for drinking purposes, Store water for drinking purposes, Fridge Tray, Water drums, Discarded canes on the walls, Other (discarded soil pots, plates, cold drinks bottle. The Highest Number of breeding site were identified in water Tank with 24% of the total breeding sites followed by Tyres and plant pots with 19.30 % &14.40% respectively. While the lowest number of breeding sites were identified in water pot for tyre i.e. 1% followed by air condition water and park bush with 1 and 2% respectively (figure No 12 and table No 5.

In our current research the survey was conducted in 4 rounds for the identification of potential breeding sites and number of larva as shown in the table no.6. The total number of houses screened were 30,406 and the total number of breeding place were 844,076 and total number of larva recovered from different sites were 206. For the outdoor potential breeding sites, 10,685 different sites were observed and the total number of Breeding sites observed were 72,521 and the total number of larva recovered from the sites were 226

Figure 12: show different potential outside Breeding Ground

Table 5: % age of potential breeding sites indoor survey

No	Name Specific Site	Number of Sites	%age
1	Water drums	140145	37
2	Water Tanks	64391	17
3	Store water for drinking purposes	26514	7
4	Discarded Material	26514	7
5	Old Tyres in the houses	22727	6
6	Construction waste	22727	6
7	Store water for drinking purposes	19772	5.22
8	Animal water utensils	7666	2
9	Standing water in the houses	11364	3
10	Air cooler Excretion water	1516	0.4
11	Mini Plant Pots	11364	3
12	Animal water utensils	3031	0.8
13	Fridge Tray	7570	2
14	Leaking Water Taps	7486	2
15	Cover of Gutter	3409	0.9
16	Discarded chains on the walls	304	0.08
17	Other (discarded soil pots, plates, cold drinks bottle etc.)	2270	0.6
	Total	378770	100

Figure 13: show different indoor potential Breeding Ground

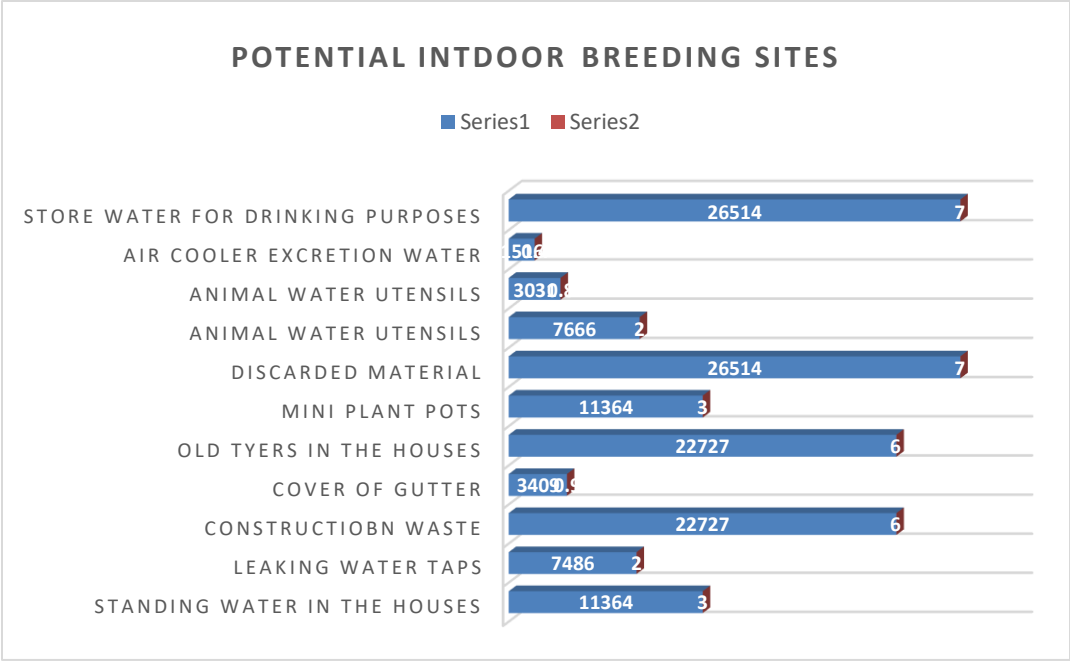

Figure 14: show different % age of potential breeding sites indoor survey

Figure 15: show searching for Recovery for larva

.

Table 6: show combine surveiillance Report of Dengue Larva.

Combined /Total Surveillance Report of Dengue Larva (Round wise)				
Indoor / Outdoor	**Activity /Round**	**Total Households / Sites Checked**	**Total Potential Breeding Sites Checked**	**Total Larva recovered Sites**
Indoor	1st Round (From 22, April to 30th April 2013)	3,407	396,052	199
	2ndRound (From 19th May to 29th May, 2013)	1,174	378,770	00
	3rdRound (From 10th June, to 22nd June, 2013)	7,075	112,733	18
	4thRound (From 4th Aug, 2013, 16nd Aug 2013)	**18,750**	**310,209**	**206**
	Total	**30,406**	**840,764**	**423**
Outdoor	1stRound (From 22 April, to 30th April, 2013)	2,867	18,067	17
	2ndRound (From 20Th May, to 29th May, 2013)	3,023	25,980	54
	3rdRound (From 10th June, to 22nd June, 2013)	2,154	12,402	18
	Special Activities (From 25th June, to 3rd Aug, 2013)	529	2,874	56
	4thRound (From 4th Aug, 2013, to 16nd Aug)	**2,112**	**13,198**	**81**
	Total	**10,685**	**72,521**	**226**

DISCUSSION

Dengue has a worldwide history of about 200-400 million mostly causing infection in tropical and sub-tropical regions. Dengue virus came to Pakistan mostly through tyre imports at Karachi ports from different world destinations. Up till now the virus infection has caused outbreak in Pakistan(Fatima, Idrees et al. 2011) In 1982, dengue was identified in Pakistan. In Punjab, out of 174 suspected patients 12 were infected by dengue virus(Fatima, Idrees et al. 2011). During 1995 only two cases of dengue infection were reported in Baluchistan (Akram et al. 1998). DENV-1 and DENV-2 were found in patients using ELISA (E khan et., 2006).

In 1985 research was conducted to identify dengue virus in Pakistan. Findings included that 20-30 % of Pakistani who lived-in Karachi were haemagglutination inhibition (Suleman, Faryal et al.) antibody positive for West Nile, Japanese encephalitis and DENV-2 Filoviruses. The reported cases of dengue infection increase from July to October due to suitable environment (Chong CF et al. 1994). In Pakistan first outbreak was reported in 1994 in which 15 patients had dengue IgM identified using DENV-2 antigen. It was also identified that 3 out 10 patients of dengue were infected from DENV-1 & DENV-2 (Paul et al. 1998)

In 1995, DENV-2 virus was identified from Baluchistan province by using ELISA test(Khan, Siddiqui et al. 2007). Our current research shows the circulation of dengue virus among the population of District Swat. In District swat 2013 dengue outbreak was the first dengue infection reported in Khyber Pakhtunkhwa in which 798 dengue infections were recorded, belonging to different area of district Swat. The infection rate was found high in urban areas as compared to rural. Out of the total reported cases of dengue infection 35% infections were observed in Saidu Sharif, 27% in Mingora city, 12% in Matta, 9% in Kanji, 4% in Fatehpur, 7% in Charbagh and 6% in Khwazakhela. In 2005 outbreak of dengue fever in Karachi, DENV-3 was reported in some patients. In serum obtained from the children in Karachi DENV-1and DENV-2 was found using serological studies (Khan E et al. 2008).

In our research the rate of infection was low in June (14.8%) due to relatively low temperature of an average 50.2 ^{0}F and gradually increased in start of the rainy season in July (17.1%) with an average temperature of 55.4^{0}F and the rate of infection was much higher (27.1%) in August with an average temperature of 48.2 ^{0}F. In September, the rate is of infection was recorded at (22.7%) due to decrease in temperature (47.3^{0}F) and

rainfall, it might be due to the well-established breeding of *Aedes* vectors for dengue virus) in raining months

DENV-2 & 3 were found to be co-circulated during 2006 in the outbreak of Karachi (Dash PK et al. 2006). In 2013 dengue outbreak in Swat out of 8927 dengue patients (male=6074 & female=2853), the rate of infection in males was high as compared to the females while a lower infection rate was observed in both males and females below the age of 20. The case confirmation was done for anti-dengue antibodies in which NS1 positive cases among population for males and females were 6074 and 2853, respectively. Dengue strips were used for the screening of the suspected patients. DENV-3 in 2006 outbreak in Pakistan was found to be closely related to DENV-3 in 2004 outbreak (Khan and Khan 2015). In 2008, a dengue outbreak was reported in Lahore infecting large number of citizens. In 2009, it was identified that the children live in Karachi have high level of anti-dengue 1gM antibody (Fatima Z et al. 2011). Some patients had co-infection with the genotype of DENV-3 & DENV-2. It showed that DENV-2 is most prevalent in the sample collected from the suspected patients. Studies showed that serotype DENV-2 was dominant in samples of dengue virus infection collected during the period of three years from 2007 to 2009 (ARY News 2010).

In the month of November 2010 outbreak, it was reported by a private news channel that out of 5,050 patients, 2,350 patients were from the province of Sindh, 1,885 from Punjab province and at 158 patients from Khyber Pakhtunkhwa province. The sample had an infection with DEN-2 and DEN-1 (ARY News 2010)

During 2011, the dengue infection rapidly assumed the proportion of endemic, specifically in province of Punjab and particularly in Lahore where, in the month of September, more than 250 people were reported dead as per data of Punjab health department over 12000 people were infected during the month of January to September 2011. In our current research the number of case of dengue haemorrhagic fever was between the ages of 15 to 30 year, in male population the rate of infection is comparatively high as compare to female. The patient expressing characteristic symptom of Dengue fever were hospitalized in Shifa Hospital Swat. In most of Dengue patient the fever was continuous lasting for 4 to 7 days while in some cases fever was remittent and intermittent. the anti-dengue antibody (NS1, IgG, and IgM) were indented while in most cases the NS1 was high rate as of other low platelet counter was also take in to account was also consider during this study from which we had come to know that during the dengue fever and dengue haemorrhagic fever, Platelet count gradually decreased, patient with low platelets count were at more severe condition.

Dengue infection often recorded in the form of huge outbreaks. There is also seasonality of dengue with the outbreak occurring in different period of the year. This seasonality is determining by the high rate of infection of disease which influence by the characteristics of the host, the vector and the agent. In the current study diagnosis of dengue virus infection was detection of dengue specified antibody. Serological test is available for dengue fever was IgM, IgG & NS1 antibodies strips which identified within 15 minutes which also help in differentiating between primary and secondary infection of dengue.

Dr. Abdul Khaliq who's is the charge of dengue unit in Swat district health department told that the number of new patients is now decreasing and the average number of new patients has dropped from 300 to 135 per day when the temperature is reduced. There have been multiple outbreaks in the country from 1994 to 2013 in different regions as given below in table

Table No 7: Detection of dengue Genotyping in different outbreaks in Pakistan from 1994 to 2013.

CONCLUSION

From our current study, we conclude the following:

Dengue infection rate is higher in Male patient as compared to Female.

The infection rate was higher among the age group of 15-30 in both male and female patients.

Platelets count decrease during Dengue infection.

Generally continuous fever ranging from 3 to 7 day were observed in dengue patients.

Highest number of outdoor breeding sites were identified in drums while indoor most breeding site were observed in pots.

In the Month of August most infections were observed due to relatively high humidity.

During the final phase of disease, the infected patients were in critical condition due to bleeding and respiratory failure.

The Signs and symptoms observed in Dengue patients were fever, vomiting, abdominal pain, splenomegaly and low platelets count.

Highest infection rate of 35% was recorded for

Saidu Sharif.

As currently no treatment/cure is available for the treatment of dengue infection, on the basis of the current study we recommend that for the prevention of dengue outbreaks, sprays at potential breeding sites both indoor and outdoor public places may be carried out to control the dengue vector breeding sites. Awareness campaigns through seminars, conference, sign boards are needed from government and non-government organizations through which the common people may be made aware to use repellent nets mosquito quills to reduce the likelihood of dengue infection by eliminating the vector.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

. The work presented in this manuscript was accomplished under the sympathetic attitude, animate directions, scholarly criticism and enlightened supervision of

Dr. Farhad Ali, Assistant Professor of department of Biotechnology, Bacha Khan University & Dr. Abdul Khaliq who's is the charge of dengue unit in Swat district health department and highly acknowledge the collaboration of Chief Executive, and Medical Superintendent of Saidu Group of Teaching Hospital Swat KPK, Pakistan.

His efforts towards the inculcation of spirit of hard work and maintenance of professional integrity besides his valuable suggestions will always serve as beacon of light through the course of my life.

AUTHOR CONTRIBUTIONS

Fawad Ali FA performed all the experiments including Laboratory activities, Farhad Ali F.A supervised and designed the study and, Muhammad Junaid Yousaf M.J.Y, and Salma Noreen SN wrote the original draft of the manuscript, Rakhshinda Sadiq R.S, Nadia Sharif N.S, Saeeda Saeeda SA, Asma Shah A.S, and Kaleem Ahmad KA. assisted, reviewed and editing the manuscript. All authors have read and agreed to the published version of the manuscript.

REFERENCES

Abbas, A., Abbas, R. Z., Khan, J. A., Iqbal, Z., Hayat Bhatti, M. M., Sindhu, Z. U. D., & Zia, M. A. (2014). Integrated Strategies for the Control and Prevention of Dengue Vectors with Particular Reference to Aedes aegypti. *Pakistan Veterinary Journal*, *34*(1).

Akram, D. S., Igarashi, A., & Takasu, T. (1998). Dengue virus infection among children with undifferentiated fever in Karachi. *The Indian Journal of Pediatrics*, *65*(5), 735-740.

Butt Q., Khan E., Rumina H., Dengue will continue to haunt Pakistan for many years 2011

Chong, C. F., Ngoh, B. L., Tan, H. C., Yap, E. H., Singh, M., Chan, L., & Chan, Y. C. (1994). A shortened dengue IgM capture ELISA using simultaneous incubation of antigen and peroxidase-labeled monoclonal antibody. *Clinical and diagnostic virology*, *1*(5-6), 335-341.

Fatima, Z., et al. (2011). "Serotype and genotype analysis of dengue virus by sequencing followed by phylogenetic analysis using samples from three mini outbreaks-2007-2009 in Pakistan." BMC microbiology **11**(1): 1-8.

Iqbal, M. P., & Qureshi, N. A. Assessing the Lack Awareness and Dangers of Dengue in Rural Punjab Pakistan.

Iqbal, M. P., & Qureshi, N. A. Assessing the Lack Awareness and Dangers of Dengue in Rural Punjab Pakistan.

Khan, E., et al. (2007). "Dengue outbreak in Karachi, Pakistan, 2006: experience at a tertiary care center." Transactions of the Royal Society of Tropical Medicine and Hygiene **101**(11): 1114-1119.

Khan, E., Hasan, R., Mehraj, V., Nasir, A., Siddiqui, J., & Hewson, R. (2008). Co-circulations of two genotypes of dengue virus in 2006 out-break of dengue hemorrhagic fever in Karachi, Pakistan. *Journal of clinical virology*, *43*(2), 176-179.

Khan, J. and A. Khan (2015). "Incidence of dengue in 2013: dengue outbreak in District Swat, Khyber Pakhtunkhwa, Pakistan." Inter

J of Fauna and Biolo Stud **2**(1): 1-7.

McAvin, J. C., Blow, J. A., Putnam, J. L., & Swaby, J. A. (2004). *Field Evaluation of a Deployable RT-PCR Assay System for Real-Time Identification of Dengue Virus*. ARMY medical research inst of infectious diseases fort detrick md

Muller, D. A., Frentiu, F. D., Rojas, A., Moreira, L. A., O'Neill, S. L., & Young, P. R. (2012). A portable approach for the surveillance of dengue virus-infected mosquitoes. *Journal of virological methods*, *183*(1), 90-93.

Paul, R. E., Patel, A. Y., Mirza, S., Fisher-Hoch, S. P., & Luby, S. P. (1998). Expansion of epidemic dengue viral infections to Pakistan. *International journal of infectious diseases*, *2*(4), 197-201.

Suleman, M., et al. (2016). "Identification of concurrent infection by multiple dengue virus serotypes during an epidemic in 2011 in Pakistan." Journal Microbiology & Experimentation **3**(3): 00088.

Thai, K. T., Phuong, H. L., Nga, T. T. T., Giao, P. T., Van Nam, N., Binh, T. Q., ... & de Vries, P. J. (2010). Clinical, epidemiological and virological features of Dengue virus infections in Vietnamese patients presenting to primary care facilities with acute undifferentiated fever. *Journal of Infection*, *60*(3), 229-237.