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## Incidence of dengue in 2013: Dengue outbreak in District Swat, Khyber Pakhtunkhwa, Pakistan

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### Abstract

This study was conducted to evaluate the incidence outbreak of “Dengue Fever” in district swat to study the Epidemiology and Clinical characteristics of Dengue fever and to conduct the population-based surveillance for determining the proportion of Dengue Virus infections, dengue incidence and disease burden. The total numbers of patients investigated were 6000 (68.6% male and 31.3% female). The male population was greatly affected (68.6%) than female (31.3%) while the infection rate was less in children. The death rate was 47 (0.78%), the infection rate was much high from 20-40 years of age (male 44.65%, 18.06% female). The characteristics symptoms observed were fever (100%), vomiting (70.18%), abdominal pain (50.16%), splenomegaly (34.16%), in majority of the patients platelet count ranged between 100,000-150,000/cmm, fever duration in most of the patients was 7-15 days.

The fever duration also varied in 1346 (22.43%) patients was observed continuous up to many days, 3880 (63.33%) intermittent and 854 (14.23%) patients were remittent. DHF is defined as acute fever, from 2 to 7 days, with hemorrhagic tendencies, for the conformation of DHF, the tourniquet test were done and also by the symptoms of patients i.e. petechiae (18.66%), and bleeding of mucosa (2.56%).

**Keywords:** Dengue Fever, Dengue Hemorrhagic fever, Epidemiology, Clinical Findings.

### 1. Introduction

The dengue virus (DEN virus); a member of the family Flaviviridae, genus flavivirus contains four antigenically related but distinct serotypes; DEN-1, DEN-2, DEN-3 and DEN-4. The incidence of disease and its severity varies between primary and secondary infections and possibly also across different dengue virus serotypes [1]. Dengue virus (DENV) infection is an important arthropod-borne viral infection which infecting about 2.5 billion people around the globe, of which approximately 975 million belong to large and small regions of tropical and subtropical countries in Southeast Asia, the Pacific and the America [2]. In recent years there is through increase observed of dengue prevalence especially in Americans, Western-Pacific and South-East-Asia [3].

Some patients with dengue fever go on to develop dengue hemorrhagic fever (DHF), which is a severe and sometimes fatal form of the disease. Around the time the fever begins to subside (usually 3–7 days after symptom onset), the patient may develop warning signs of severe disease. These signs include severe abdominal pain, persistent vomiting, marked change in temperature (from fever to hypothermia), hemorrhagic manifestations, or change in mental status (irritability, confusion, or obtundation). The patient also may have early signs of shock, including restlessness, cold clammy skin, rapid weak pulse, and narrowing of the pulse pressure. Patients with dengue fever should be told to return to the hospital if they develop any of these signs. A dengue epidemic requires the presence of the following three main factors [4].

- The vector mosquito (primary *Aedes aegypti*).
- The dengue virus.
- A large number of susceptible human hosts.

Outbreaks may be explosive or progressive, depending on the density and efficiency by which the vector can be infected, the serotype and strain of the dengue virus, the number of susceptible or non-immune humans in the population, and the amount of vector-human contact [5]. It is thought that in last 50 years, its incidence has increased 30-times more with increasing geographic expansion to new countries and, in the present time, from urban to rural areas [6]. The number of cases reported annually to WHO are ranged from 0.4 to 1.3 million in the decade 1996-2005. As an infectious disease, the number of cases varies substantially from year to year [7].

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*Aedes* mosquitoes are the principle vector in the transmission of disease. This special mosquito is well adapted to humans and prefers to live in clean surroundings in close proximity to clean human surroundings [8]. Dengue virus serotypes were classified by Albert Sabin in 1944. Infection with DEN viral serotypes has been a major concern in DEN endemic areas worldwide. The reason behind this is the co-circulation of all the four serotypes of the dengue virus [9].

Dengue virus presently threatens half of the world's population and is an important public health problem in many tropical regions of the world [10]. Geographic distribution and genetic diversity of dengue virus suggests its origin in Asia. The first reported out-break of DHF was from Philippine in 1953 [11]. In the last three decades, the demographic and clinical features of dengue infections have changed rapidly. Pakistan experienced the first major outbreak of Dengue in 2004 and the serotype identified was DV-2 [12]. Thereafter, several studies from Pakistan and other endemic areas reported DV-1 and DV-2 to be the predominant serotypes in circulation among population [13].

Based on data from 112 national vital registration systems, 12,000 deaths in Southeast Asia, 4,000 in Western-Pacific and 2,000 in America for the year 2002 have been estimated due to dengue fever [14]. In Asia, dengue has made its route geographically from Southeast Asian countries [15]. Since then, multiple outbreaks have been reported from different regions of India [16]. This was followed by the introduction of DV-3 in our population in 2005 resulting in another epidemic [17]. During the most recent epidemic in 2006, the genotypes DV-2 and DV-3 were found to be prevalent in Pakistan [18]. The epidemics of dengue have been commonly associated with the rainy season [19]. The peak incidence of dengue fever was reported to occur from August to October in Pakistan [20].

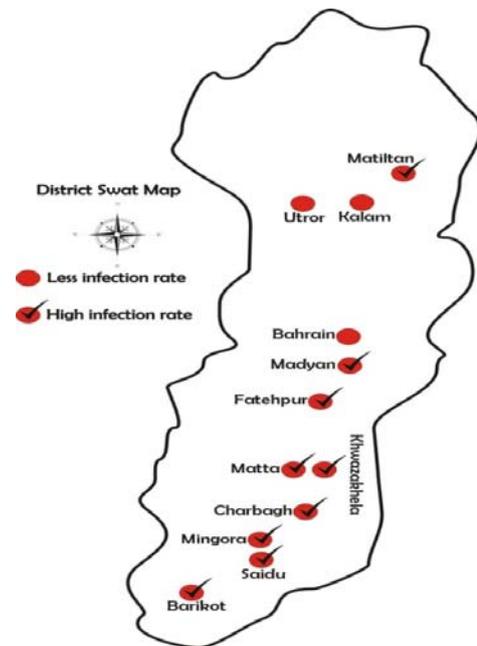
Pakistan has experienced a number of dengue fever outbreaks since 1992. In the 2005 Karachi outbreak 4,500 dengue cases were identified which affect a large population of people in Azad Jammu & Kashmir in 2006 but went largely unreported more than 21,204 people were reportedly infected in whole country in 2010 [21]. In year 2011 the massive outbreak in Punjab attracted the attention of the Government of Pakistan, especially the Punjab Government [22]. In Pakistan the first outbreak of DHF was reported in 1994 which was confirmed by the Aga Khan University Hospital (AKUH), the serotype was DENV-2 [23]. During 2005—2006, there was an unprecedented increase in epidemic DHF activity in the country, a large number of cases were reported from Karachi. In which more than 3640 patients with signs and symptoms of DF were admitted to several referral hospitals in the country, including the Aga Khan University Hospital AKUH. There were 40 deaths occurs, in which 37 were from Sindh province, considering the largest and most severe outbreak of DF in the Pakistan [24]. In 2013 Dengue fever outbreaks reported from the less endemic areas in Pakistan. A huge Dengue fever outbreak is being confronted in district Swat of Khyber Pakhtunkhwa province. In district Swat from 7th August to 25th September 2013 total 6,376 Dengue fever cases and 23 deaths have been reported from this area [25]. The contributing factor in the predisposition of disease includes the age of patient [26]. Immune status, type of virus infecting, humid climates especially rainy season and temperature >20 °C. The residents returning from overseas countries which are dengue endemic, increase in population growth, unplanned urbanization, increase in the international trade and increase in air travel all of these provide an ideal way of transporting virus

to new areas [27].

## 2. Methods & Materials

### 2.1 Description of the Study area

The lush green and historic Swat Valley lies between 34°-40' to 35° N latitude and 72' to 74°-6' E longitude and is part of the Provincially Administrated Tribal Area (PATA) of the Khyber Pakhtunkhwa Province of Pakistan. Total area is 5,337 km<sup>2</sup>, total population is 1,257,602 and capital is Saidu Sharif. The valley is an integral part of the strategic and significant region where three parts of the Asian continent—South Asia, Central Asia and China, meet.



Nine regions of district Swat were brought under considerations which were adversely effected by dengue virus including Mingora, Saidu Sharif, Warkay, Barikot, Matta, Matiltan, Charbagh, Manglawar & Salampur. The research was carried out with the collaboration of Saidu Teaching Hospital, Shifa Medical Center and Saidu Sharif Swat in five months period of July-November 2013. During this visit to different hospitals five ml blood was collected from each patient along with a proforma containing the whole information of respective patients. The blood samples were immediately brought to the Zoology department Abdul Wali Khan University Mardan (AWKUM), Buner Campus, Khyber Pukhtunkhwa. The serum was isolated from the blood in eppendorp tubes through centrifuge machine and preserved at -80 °C.

### 2.2 Collection of Clinical Data

The data regarding signs, symptoms and laboratory analysis was collected from the admitted patients who presented dengue infection symptoms and fulfilling the diagnostic criteria of dengue fever and dengue hemorrhagic having positive IgG, IgM, NS1 anti-dengue antibodies. All the infected patients were examined thoroughly for Petechiae, Abdominal pain, Enlarged liver (splenomegaly), Epistaxis, Gum bleeding, Haematemesis, Loose motion, vomiting. The analysis performed were platelets count, liver function, and anti-dengue antibodies NS1, IgM, IgG with the help of different diagnostic tools.

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**3. Results**

**3.1 Epidemiological findings**

We investigated 6000 cases of Dengue fever & DHF patients out of which 4121 (68.73%) were male and 1879 (31.31%) females. most of the patients were belonging to the urban areas i.e. Mingora, Saidu Sharif, Watkay, Barikot & Matta while some patients belong to rural areas of Matiltan, Charbagh, Manglawar & Salampur of district Swat. Most of the effected patients were in age of 21-40 years. Figure 1.0 shows the age and sex wise distribution ratio of dengue infected patients.

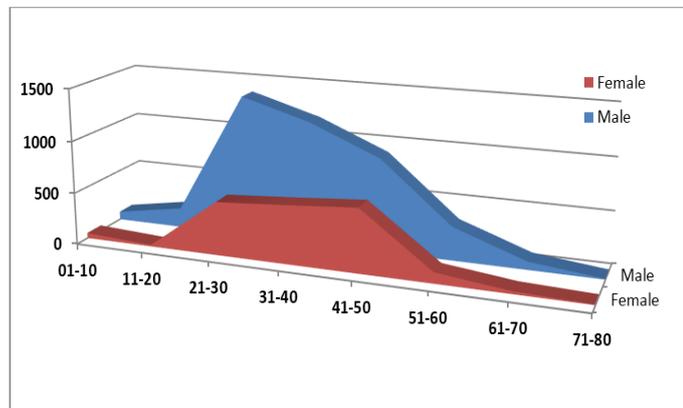


Fig 1.0: dengue infection ratio among the patients n=6000

**3.2 Dengue infection among population**

Among the nine infected areas the rate of infection was high in urban areas as compared to rural, in Saidu infection was observed (35%), Mingora city (27%), Matta (12%), Kanju (09%), Patehpur (04%), Charbagh (07%) and Khwazakhela (06%).

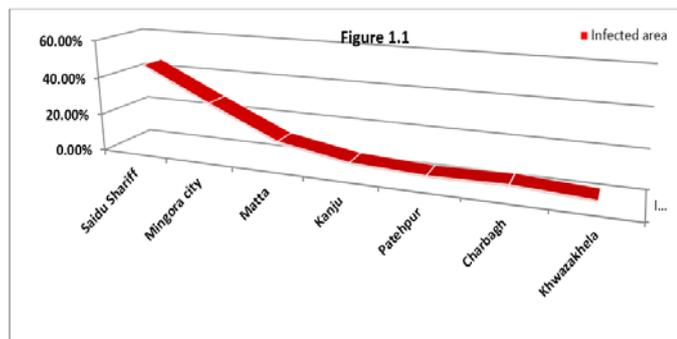


Fig 1.1: Show the infection rate among different areas of district swat

**3.3 Clinical findings**

The most common symptoms among the infected patients 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 ( See Figure 2.1).

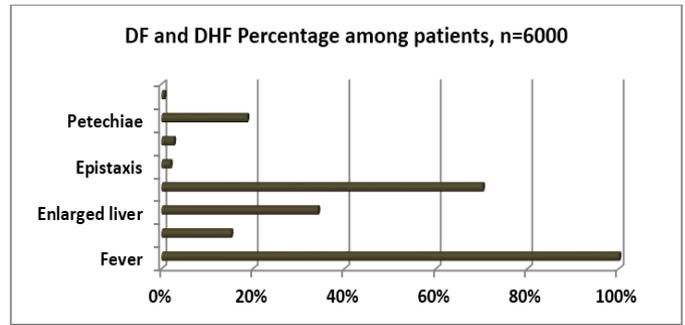


Fig 2.1: Patients presenting signs & symptoms of dengue fever (n=6000)

During the 5 months study patients identified with dengue fever and DHF in which the condition of fever in 1346 (22.43%) patients were found continuous up to many days, 3880 (63.33%) was presenting intermittent and 854 (14.23%) patients were remittent. As shown in Figure 2.2.

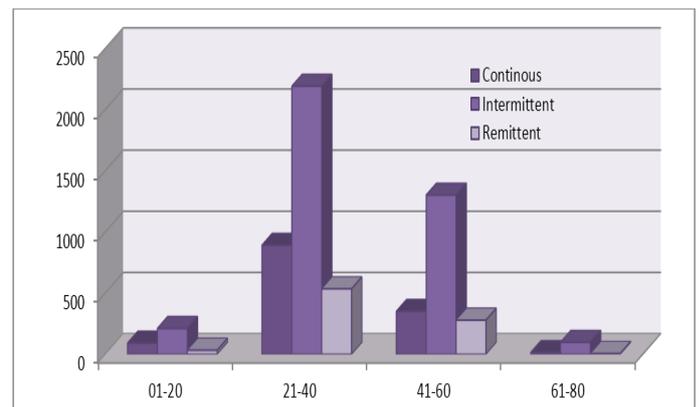


Fig 2.2: Duration of fever of dengue patients (n=6000)

Figure 2.3 showing anti dengue antibodies among all the patients in which 4121 were male showing NS1 (3359) and IgG, IgM (762) and of females NS1 (1171) and IgG, IgM (708) was investigated respectively.

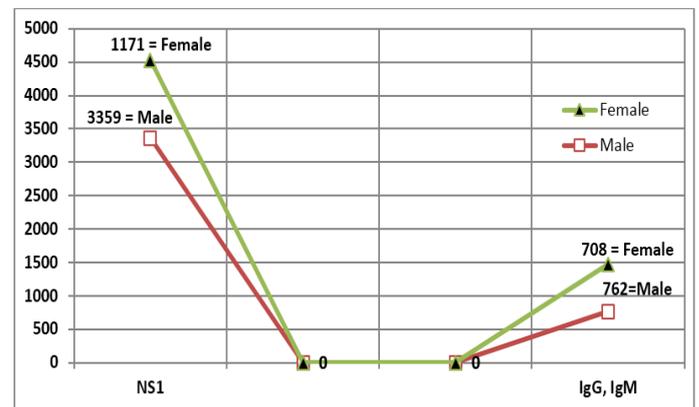


Fig 2.3: showing Anti Dengue Antibodies in patients (n=6000)

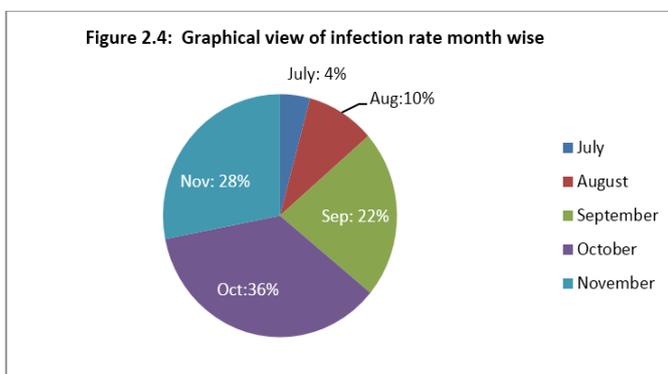
The platelets count in 910 (15.16%) patients was less than 50,000/cmm, in 1719 (28.65%) patients it range from 50,000-100,000/cmm while in 3371 (56.18%) patients the platelets count was from 100,000 to 150,000/cmm.

The rate of infection was low in July and gradually increased in rainy months and the rate became much high in September and October (Table 2.5), it might be due to the well-established breeding of *Aedes* (vectors for dengue virus) in raining months.

**Table 2.1:** Table showing the infection of dengue infection month wise (n=6000)

July	August	September	October	November
250 (4.16%)	571 (9.51%)	1345 (22.41%)	2167 (36.11%)	1691(28.18%)

In July the rate of infection was less (4.16%), while it gradually increases in August (9.51%), during September (22.41%) and October (36.11%) the infection rate of DF and DHF rapidly increases due to monsoon season, while in November (28.18%) there is a clear decrease which can be seen from below figure no 2.4.



**Fig 2.4:** Graphical view of infection rate month wise

#### 4. Discussion

In 1995, DEN-2 infection was reported from Baluchistan province [28]. In 1998 from Baluchistan, DEN-1 and DEN-2 were found in patients by using ELISA study [29]. The current study shows the circulation of DENV-2 and DENV-3. Dengue has a worldwide history of about 200–400 years, mostly causing infection in tropical and sub-tropical areas. Dengue virus is believed to come in Pakistan with tyres at Karachi sea port carrying eggs of infected mosquitoes. To date, dengue virus infection has caused several outbreaks in Pakistan [30]. Until 1994, there was no valid data available about dengue infection cases in Pakistan. In 1982, Dengue was identified in Pakistan, Punjab in which out of 174 patients, 12 were dengue virus positive [31].

In 1985, a research was conducted to study the prevalence of dengue virus infection in Pakistan. It showed that about 50–60% of the Pakistanis, especially those living in Karachi were haemagglutination inhibition (HI) antibody positive for West Nile, Japanese encephalitis and DENV-2 Flaviviruses. These cases rapidly increased from July to October in patients ranging from 6 to 20 year age [32]. In 1994, first outbreak of DHF was reported in Pakistan, out of 16 patients, 15 had dengue IgM identified using DENV-2 antigen. It was also observed that in three out of ten patients of dengue virus were infected with DEN-1 and DEN-2 [33]. In 1995, DEN-2 infection was reported from Baluchistan province [34]. In 1998 from Baluchistan, DENV-1 and DENV-2 were found by using ELISA study [35]. The current study shows the circulation of DENV-2 and DENV-3. This 2013 dengue outbreak in district

swat is the first reported outbreak in literature in Khyber Pukhtunkhwa, in which more than 8000 dengue incidences were recorded from different areas of this region, among the nine infected areas the rate of infection was found high in urban areas as compared to rural, in Saidu infection were observed (35%), Mingora city (27%), Matta (12%), Kanju (09%), Patehpur (04%), Charbagh (07%) and Khwazakhela (06%).

In 2005, outbreak of DHF in Karachi, DEN-3 was reported among the few tested patients. In serum of children in Karachi, DEN-1 and DEN-2 was found using serological studies [36]. DEN-2 and DEN-3 were found to be co-circulated during 2006 outbreak in Karachi [37]. Similarly in 2013 dengue outbreak in swat, out of 6000 dengue patients (male=4121 & female=1879), the male infection rate was found more as compared to female, the cases confirmation was done for anti-dengue antibodies in which NS1 positive cases among population were male=3359, female=1171, while IgG, IgM observed were male=762, female=708. The positive blood samples were subjected to RT-PCR method, the serotypes present in this outbreak were found DENV-2 & DENV-3 respectively, which shows similarities to 2005 dengue outbreak during Karachi because of the same serotypes detected in the blood of patients.

DENV-3 in 2006 outbreak in Pakistan was found to be closely related to DEN-3 in 2004 outbreak in New Delhi [38]. In 2008, a dengue outbreak was reported in Lahore infecting large number of citizens of Lahore. Samples were found to have DEN-4, DEN-2 and DEN-3 infection [39]. In 2009, it was reported that children living in Karachi had high levels of anti-dengue IgM antibody [40]. Samples had concurrent infection with serotypes DEN-2 and DEN-3. Studies showed that serotype DEN-2 was dominant in samples of dengue virus infection collected during the period of three years from 2007 to 2009 [41].

During 2013 dengue outbreak in swat district DENV serotypes 2 & 3 were found in which the serotype type 3 was found more prominent (50%) and type-2 (39%). The same results were also investigated by Aga Khan University Hospital Karachi during the 2005 outbreak in which they stated that the DENV type-2 and type-3 were more prominent as compared to DENV type-1. This outbreak shows similarity to 2005 outbreak during Karachi.

In November 2010 outbreak, it was reported by a private news channel that out of the 5,050 patients, 2,350 patients were from Sindh, 1,885 from Punjab and at 158 patients from Khyber Pakhtunkhwa. The samples had an infection with DEN-2 and DEN-1 [42]. During 2011, the disease rapidly assumed the proportions of epidemic, specifically in Punjab and particularly Lahore where, in September, more than 250 people were reported dead and, according to the Punjab Health Department; over 12,000 people were infected during January to September 2011.

In our study the number of cases of DF & DHF was among the age of 21-40 years of age. Male population infection rate was much high, while in female the ratio was low, the patients presenting the signs & symptoms were hospitalized in Saidu Teaching Hospital & Shifa Medical Center. The duration of fever was observed between 4-7 days, most of the patients presented the remittent duration of fever. The anti-dengue antibodies (NS1, IgG, and IgM) were observed while in most cases the NS1 was at high rate as of others. Low platelets count was also take into account during this study from which we had come to know that during DF & DHF infection the

platelets count gradually decreased, patients with low platelets count were at more severe conditions.

It is clear that dengue cases show cyclical variation with high epidemic years and non-epidemic years. Dengue often presents in the form of huge outbreaks. There is, also a seasonality of dengue, with outbreaks occurring in different periods of the year. This seasonality is determined by high transmission of this disease, which is influenced by characteristics of the host, the vector and also the agent. The serotypes responsible for disease were identified as DENV-2, & DENV-3 circulating in the year in the population.

In the current study, diagnosis of dengue virus infection was primarily based on serology via detection of dengue specific antibodies. Serological tests available for dengue fever were IgM, IgG & NS1 antibodies strips which detect within 15 minutes of duration which also help in differentiating between primary and secondary infections of dengue.

Regarding the circulating DENV serotypes (DENV-2 & DENV-3) in the 2013 dengue outbreak in Swat, our result is also confirmed and authenticated by a report of WHO about the circulating serotypes of dengue virus (DENV-2, DENV-3) in different patients from Swat [43]. While about the history of infection in this outbreak, few cases of dengue fever have been diagnosed with no travel history out of the district most probably the infection was acquired locally.

According to verbal report of Dr. Haider Ali, who is in charge of the dengue unit at the Swat District Health Department, Twenty-nine people had died up to the end of October 2013, more than 8,920 were tested positive, and the district's health officer, Dr. Abdull Khaliq also 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 reduced. There have been multiple outbreaks in the country from 1994 to 2013 in different regions as given below in table 3.2.

**Table 3.2:** showing detection of dengue serotypes in different outbreaks in Pakistan from 1994 to 2013

Outbreak Area	DENV-I	DENV-II	DENV-III	DENV-IV	Year
Karachi	x	√	x	x	1994 [44]
Karachi	√	√	x	x	1995 [45]
Balochistan	√	√	x	x	1998 [46]
Karachi	√	√	√	x	2005 [47]
Karachi	x	√	√	x	2006 [48]
Lahore	x	√	√	√	2008 [49]
Karachi	x	√	√	x	2009 [50]
Sindh	√	√	x	x	2010 [51]
Lahore	x	√	√	√	2011 [52]
Karachi	x	√	√	√	2012 [53]
Swat	x	√	√	x	2013 [54]

## 5. Conclusion

All the blood samples were confirmed through IgG, IgM & NS1 strips. The positive samples were run further for serotyping as a result dengue virus type 2 and 3 were detected. More than one DENV types detected in the population indicates that there could have been more chances of DHF and Dengue Shock Syndrome in the region. Under suitable environmental conditions, the disease can re-emerge in the coming future.

During the febrile phase of illness the dehydrating patient were in serious condition. The cause of death in most patients was pulmonary hemorrhages and respiratory failure. More cases of DF and DHF were seen in adults, especially among the patients of 20-40 years of age. despite the limitation in terms of patient population (all patients were not included) and study design, the results of our study have highlighted significant findings, such as abdominal symptoms and low platelets count in case of DF and DHF, at the time of presentation of our study among population. According to the previous dengue outbreaks in the country and from our study we conclude that Pakistan is now endemic in dengue, the serotypes (2 & 3) are responsible for the infections in 2013 dengue outbreak in the study area.

For the prevention of such types of outbreaks public awareness campaigns are needed through which we can aware the people to use mosquito repellent nets, mosquito quills, mosquito repellents in order to reduce the chance of infection by eliminating the vectors. Family support for the infected patients is also needed; fluids should be given orally if they do not tolerate the fluids orally intravenous fluids are to be given.

## 6. Competing Interest

The authors of this research declared that they have no competing interest.

## 7. Acknowledgement

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