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Chapter 7. THE EPIDEMIOLOGICAL STUDIES OF DENGUE FEVER IN KOHAT

7.1. Introduction

Dengue fever (DF) is a frequently spreading vector-borne viral infection reported throughout the world, especially in tropical and subtropical regions [1]. DF is considered a fast-spreading vector-borne infection of the 21st Century in developing countries [2]. The etiological virus of dengue fever belongs to the *Flaviviridae* family and genus flavivirus which is a single-stranded enveloped virus transmitted by the bite of female mosquitoes *Aedes aegypti* and/or *Aedes albopictus* [3]. Molecular analysis of the dengue virus reveals 4 diverse serotypes DENV-1, 2, 3, and 4 based on antigenicity [4]. Although DENV infection is asymptomatic in the majority (70%) of the cases [5] but a very small proportion (0.5 to 5%) develop severe symptoms [6]. Characteristic mild symptoms of this arboviral infection are nausea, joint pain, skin rashes, retro-orbital pain, and cough [7] while it can lead to subsequent thrombocytopenia resulting in hemorrhagic fever of severe type, and/ or a life-threatening shock [8]. Mortality rates of dengue are usually very low in the range of 1–5% [9] but it can be reduced to 1% with the proper preventive and treatment measures. Mortality rates can rise up to 26% in case of severe dengue infection [1].

Meteorological factors like temperature, rainfall, and humidity favor the circulation and persistence of DENV vectors [10–12]. In Pakistan, a dengue-like illness was first reported in 1982 in the Punjab province followed by several epidemics reported over

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decades with numerous serotypes circulating in the country [13]. In Khyber Pakhtunkhwa (KPK) the first outbreak of dengue fever was reported in 2013 in district Swat with 8546 cases and 33 mortalities. Serotypes 1, 2, and 3 were prevalent among positive cases [14]. Lately, in 2017, there was another massive outbreak of DF in the provincial capital, Peshawar [15]. Several districts in KPK faced small to medium outbreaks of DF in the past few years i-e Abbottabad with 69% prevalence, Malakand with 35%, Haripur with 30%, Sawabi with 28%, and Peshawar with 21% [8] Published literature indicates that most outbreaks were reported during or after the monsoon season [16]. However, the incidence or prevalence of DF in several potentially endemic regions of the KPK province and particularly the Southern districts such as Kohat is largely unknown thus making the dengue eradication strategy ineffective.

This study was designed to figure out dengue hotspots in district Kohat by figuring out the incidence rate of the disease in two major healthcare facilities where most of the people report from all over the district.

7.2. Experimental

7.2.1. Description of the Study Area

Kohat is situated in the South of Khyber Pakhtunkhwa (KP) province of Pakistan which is one of the four provinces of the country (Fig. 9). Kohat city is the capital of the district and 4^{th} largest city of the KP province with an area of 2,545 square kilometers (983 sq mi). The population of the district is approximately 228,779. The climate of Kohat is warm and semi-arid and the temperature rises above 37°C after Apriland reaches 42–43°C in June and July (Table 2).

7.2.2. Study Type and Designing

The presented explanatory and analytical study were premeditated in the Department of Biosciences, Molecular virology laboratory, COMSATS University Islamabad (CUI) and conducted in the district headquarters hospital (DHQ) Kohat and combined military hospital (CMH) Kohat, from July 2018 to November 2019. Before approaching the patients, consent was taken from the administration of the hospital. It was revealed by pilot studies that some areas of Kohat are at higher risk for dengue infection.

7.2.3. In Patient Department (IPD) Patients

Clinically positive diagnosed cases of dengue fever admitted in wards were directly observed and interviewed systematically. A pre-experienced questionnaire was used to gather the figures concerning platelets count, demographic variables, empty plot, territory portion, pond around the house (probable vector breeding site), house location, windows screening, and uncovered water containers in the house.

7.2.4. Sample Collection from Out Patient Department (OPDs) Patients

A total of 1665 suspected patients were interviewed from OPDs of both institutes. Suspected patients with the symptoms of high-grade fever from 3–5 days, severe headache, joint pain, skin rashes, shortness of breathing, and cough referred to a laboratory by physicians were taken in this study for specimen collection. A preceding approval was taken from each patient and in the case of a child, his/her guardians were consulted.

7.2.5. Sample Size and Laboratory Processing from OPD Patients

A total of 4 ml fresh blood specimen was obtained from each OPD patient in strictly sterile conditions and transferred to EDTA tubes for the detection of NS-1, IgG, and IgM antibodies using ELISA according to the guidelines of [17] (PLATELIA Bio- Rad lab, France). Samples were labeled with patient name, gender, age, region, and other useful information. The collected blood samples with data like patient name, age, date/month, gender, institute name along with other useful information were noted and samples were stored at -20°C until further use. Samples were further evaluated for centrifugation to isolate the serum. All sample reagents were liquefied at room temperature.

7.3. Lab Tests

7.3.1. Dengue Nonstructural protein 1 (NS1) Antigen

Initially, for rapid detection of NS1 (Non-structural 1 protein) NS1 antigen SD BIOLINE Dengue Duo rapid test kit was used following the manufacturer's protocol [17]. The serum of suspected patients was subjected to this method for the detection of NS1 antigen.

7.3.2. Dengue IgG/IgM

The serum was further subjected for the detection of IgM and IgG antibodies against DENV in the human serum. The isolated serum was sunk on the applicable spot on the kit along with the buffers and left for 15 minutes, the immunocomplex contacts non-mobile antibody results in the production of red purple color.

7.3.3. Demographic Profile

Consulted patients of OPD were determined demographically using a questionnaire during the time of sampling while IPD data were collected directly. Data concerning demographic and clinical information were gathered like age, signs and symptoms, residence area, month, and season of onset etc.

7.4. Data Analysis

All the results data are determined by frequency, percentage and graphical representation.

7.5. Results

From July 2018 to November 2019, a total of 1742 suspected samples were screened for detection of DENV, which revealed 453 positive patients with a percentage of 26% that comprises 319 males and 134 females' population. Most of the DENV cases reported in DHQ hospital (Table 1, Figure 1).

Table 1

Dengue infection Characteristics

Constraints		Incidence	Percentage
Institute	DHQ	286	29.21
	CMH	167	21.88
Gender	Male	285	62.91

continue table 1

	Female	168	37.09
Age group	05-10	59	13
Constraints		**Incidence**	**Percentage**
	10–20	134	29.58
	20–30	173	38.18
	30–45	87	19.24
Areas	Lachi	208	45.91
Shakardara	110	28.24	
	Hangu	79	17.43
College Town	35	7.72	
	Kaghazai	21	4.63

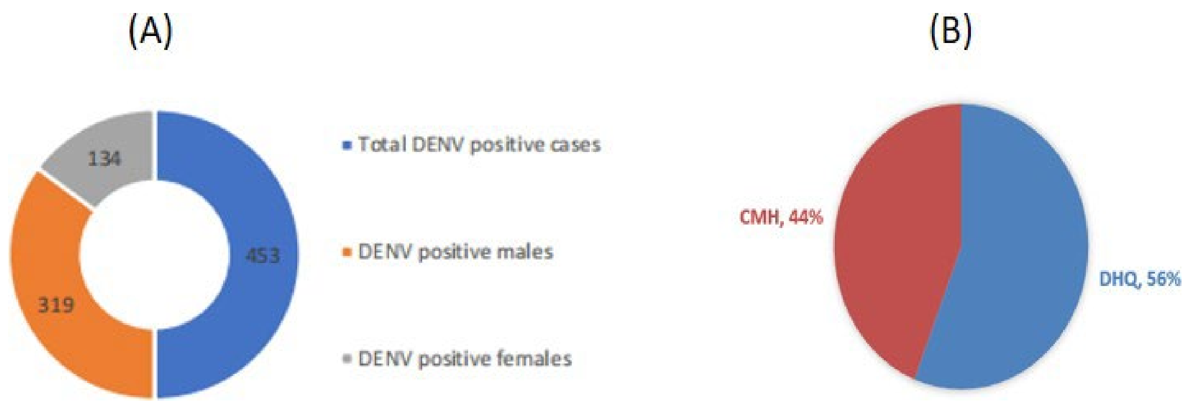

Fig. 1. The number of total cases and dengue fever positive (A). Institute-wise distribution of dengue cases (B). We can see that DHQ received more patients during the study time

Rys. 1. Liczba wszystkich przypadków gorączki denga (A). Rozkład przypadków dengi pomiędzy CMH a DHQ (B). Można zauważyć, że w DHQ odnotowano większą liczbę pacjentów

7.5.1. Laboratory markers of dengue virus infection

All of the dengue fever infected patients were found with IgG and IgM antibodies and NS1. Some patients were diagnosed with positive with IgG and IgM antibodies both. However, the incidence of IgG (47%) and IgM (27%) antibodies in infected patients were high compared to NS1 (14%) as shown in Figure 2.

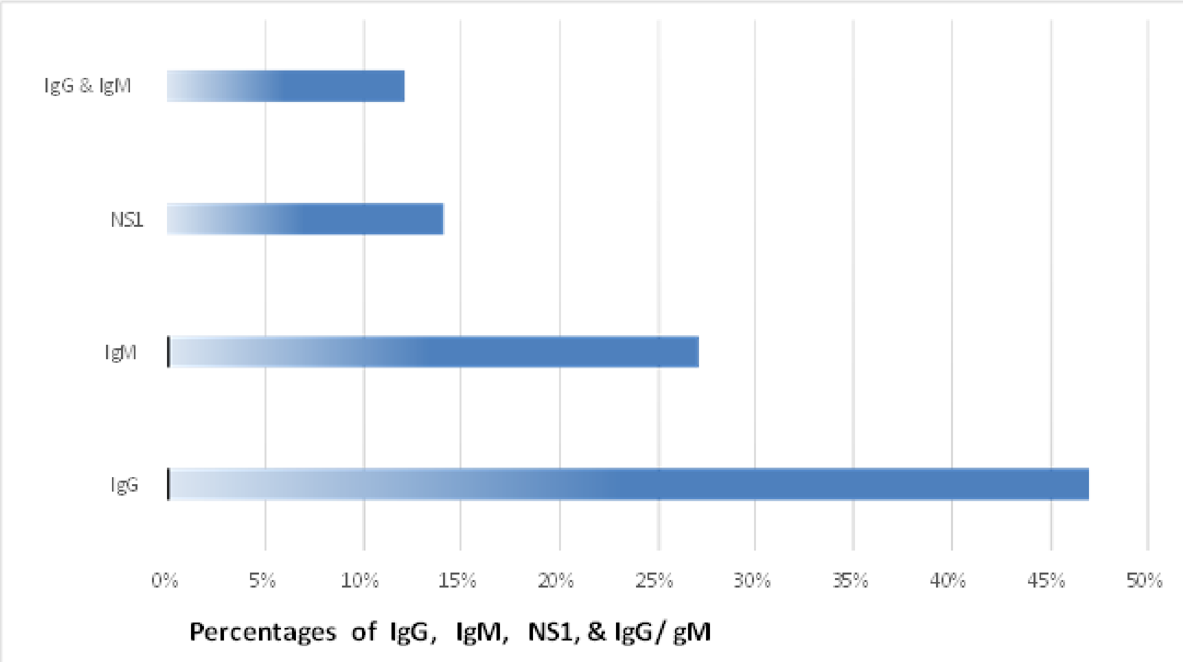

Fig. 2. Detection of DENV. It can be seen that IgG is detected in majority of the cases. Other antibodies like IgM, NS1 and others are comparatively low

Rys. 2. Detekcja DENV. IgG zostało oznaczone w większości przypadków. Inne przeciwciała takie jak IgM, NS1 oraz pozostałe odnotowane są na niskim poziomie

7.5.2. Age-Wise and Region-Based Prevalence of DENV

Results data revealed age groups 10–20 and 20–30 are more susceptible to dengue infection, the prevalence was 29.58%, 38.18% respectively. Similarly, age group 1–10 was less affected (13%) by infection while the age group 30–45 (19.24%) appeared moderately affected. (Table 1).

7.5.3. Geographical Distribution

In the existing study dengue infection mostly affected rural areas people that are living in high cultivation areas (Figure 3).

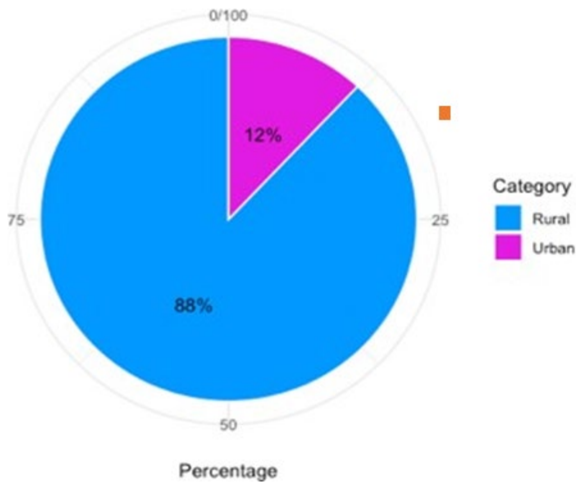

Fig. 3. Geographical Distribution of DENV. Rural regions have more DF cases compared to urban. This can be attributed to vector species distribution in urban regions and more suitable breeding places for them

Rys. 3. Geograficzny rozkład DENV, Regiony wiejskie odnotowują większą liczbę przypadkow niż regiony miejskie. Może to być związane z rozmieszczeniem gatunków-wektorów i bardziej odpowiednimi dla nich miejscami lęgowymi

7.5.4. Monthly Distribution of DENV

Data analysis revealed the incidence started in July and the highest prevalence of dengue infection was observed in October (164) (36.20%) and September 128 (28.25%). Subsequently august and July showed 82 (18.10%), and 63(13.90%) cases respectively. The percentage of infection was quite low on November 16 (3.53%) (Figure 4).

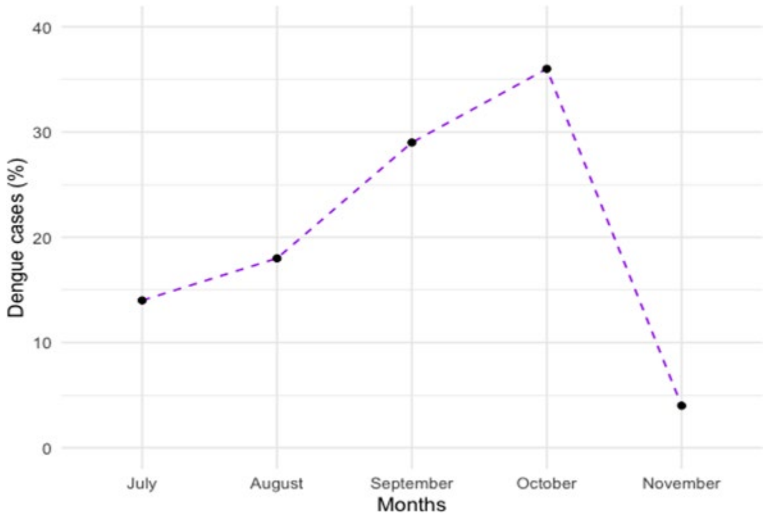

Fig. 4. Monthly prevalence of DENV infections. During the month of October, the weather remains favorable for mosquito breeding. During this month, the weather is not severe, therefore, its suites mosquito breeding

Rys. 4. Miesięczna częstość występowania zakażeń DENV. W październiku pogoda sprzyja rozmnażaniu się komarów. W tym miesiącu pogoda sprzyja rozmnażaniu się komarów

7.5.5. IPD and OPD patients of both hospitals

Among infected patients, only 77 (17%) patients were admitted to wards that were diagnosed with severe dengue infection with extreme symptoms. These pateints had low platelet count, noticeably all of the IPD patients came from Lachi and Shakaradara (cf. Figure 5).

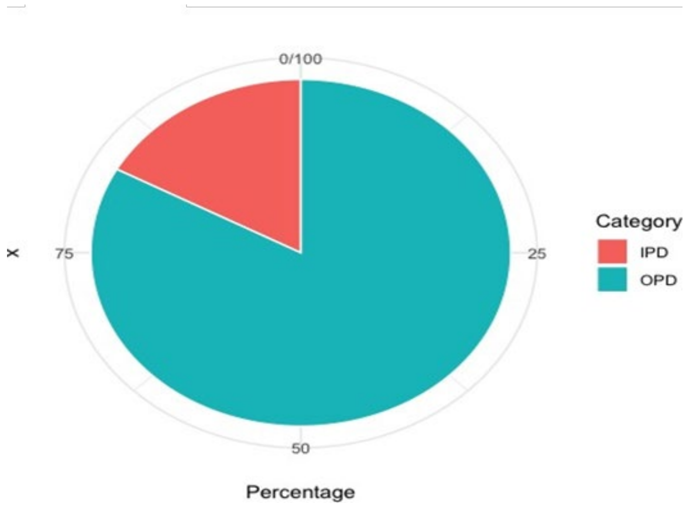

Fig. 5. Prevalence of DENV among IPD and OPD patients. OPD receives, generally more patients compared to IPD. Therefore, the incidence of DF in OPD patients is high

Rys. 5. Rozpowszechnienie DENV wśród pacjentów z IPD i OPD. OPD otrzymuje na ogół więcej pacjentów w porównaniu z IPD. Dlatego częstość występowania DF u pacjentów z OPD jest wysoka

7.5.6. Medical Records of Infected Patients

Here we present IPD patients symptomatically. All of them were observed with mild symptoms like fever, body aches, and joint pain especially in knees and shoulder joints while nausea, vomiting, and diarrhea were observed in 75% of patients, shortness in breathing (38%), and gum bleeding (7%).

7.5.7. Seasonal Deviation of Dengue Infection

The incidence rate varied with seasonal fluctuations. Monsoon sessions have a huge impact on dengue fever concerning mosquito breeding and other activities. We saw that 64.45% of cases were reported in the post-monsoon season (Figure 6).

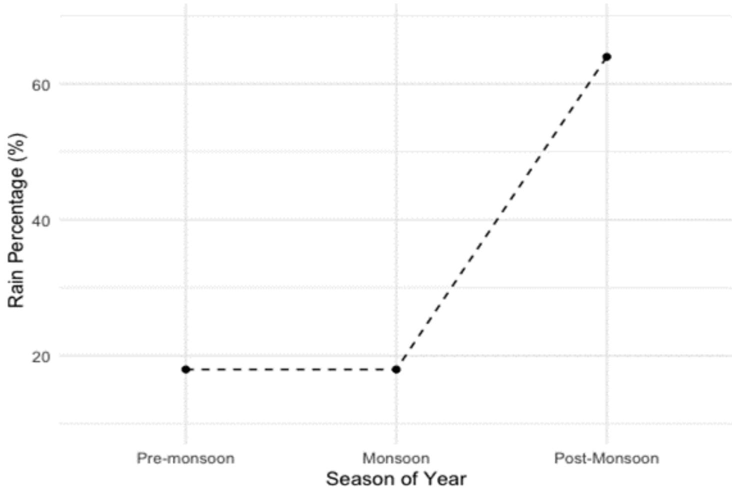

Fig. 6. Seasonal variations of dengue fever. Pakistan receives a lot of rains during monsoon, and vector breeding sites which are mainly stagnant water bodies, can be expected in great numbers post-monsoon. That is the reason why we see an exponential increase in dengue cases post-monsoon

Rys. 6. Sezonowe zmiany gorączki denga. W Pakistanie odnotowuje się wysokie opady deszcze, zwłaszcza podczas monsunów. Powstające w ten sposób stojące zbiorniki wodne są miejscem rozmanażania komarów. To jest powód, dla którego obserwujemy wykładniczy wzrost przypadków dengi po monsunie

7.5.8. Temperature Fluctuations in Kohat throughout whole year

Weather reports were scrutinized to check the impact of temperature on infection patterns. The highest temperature 44°C were reported in July month (Table 2) [17].

Table 2

Temperature of Kohat

Month	**Temperature**
January	09–20°C
February	11–22°C
March	11–32°C
April	21–36°C
May	31–42°C
June	37–43°C
July	33–44°C
August	30–38°C
September	29–39°C
October	26–39°C
November	16–28°C
December	15–26°C

7.5.9. Evaluation of Platelets Count

Thrombocytopenia, or low platelet count is one of the basic indicators of DENV infection. The total number of platelets decreases due to its destruction while fighting infection and the bone marrow fails to produce new [18]. Patients with severe symptoms were evaluated for platelet count (Figure 7). We can see that it was very low (20000 to 59000/µL) in the majority of the patients (50%).

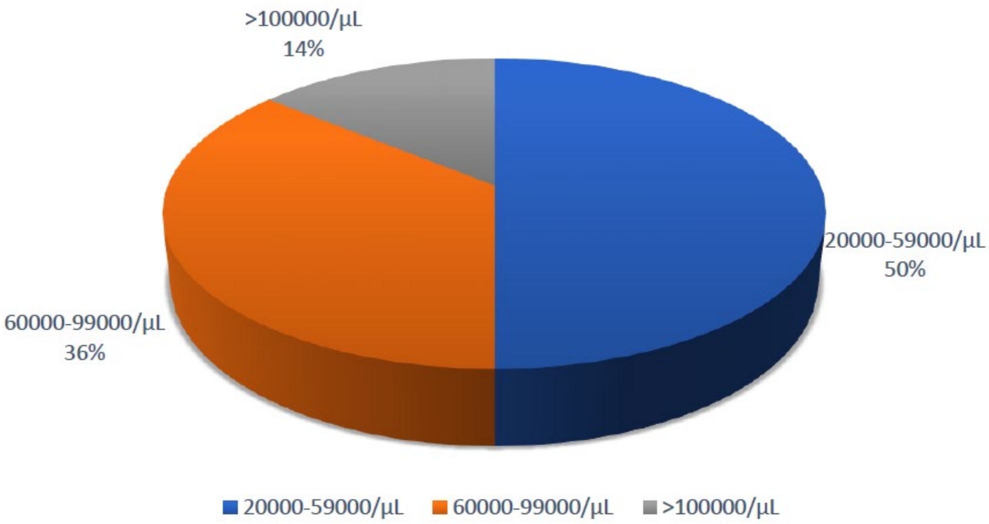

Fig. 7. Platelets count of IPD Patients. Platelets count is an important indicator of DENV infection. We can see that the majority of patients have platelets count between 20 and 59 thousand/µL
Rys. 7. Liczba płytek krwi u pacjentów z IPD. Liczba płytek krwi jest ważnym wskaźnikiem zakażenia DENV. Większość pacjentów ma liczbę płytek krwi między 20 a 59 tys./µL

7.5.10. Literacy Level of Patients

Among patients included in our study, 33% were illiterate, 25% were matric, 34% were matriculated and high school students while undergrad/graduate students were only 8%.

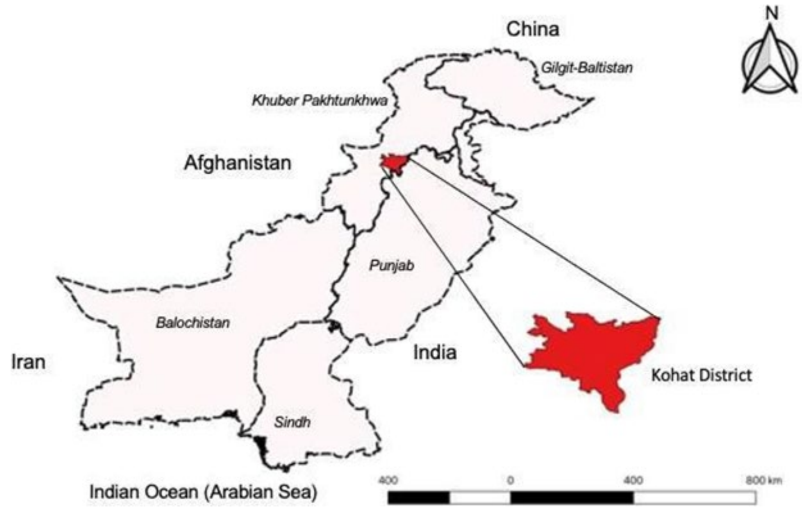

Fig. 8. Pakistan and its neighboring regions, study area (Kohat District) is highlighted as red and magnified. Kohat district lies in the Northwestern region of the country called Khyber Pakhtunkhwa. The map is generated with a free, open – source software qGIS (ver 3.28) (GNU General Public License), developed by the Open-Source Geospatial Foundation Project (http://qgis.org)

Rys. 8. Pakistan i sąsiednie regiony, obszar badań (dystrykt Kohat) zaznaczono na czerwono i powiększono. Dystrykt Kohat leży w północno-zachodnim regionie kraju zwanym Khyber Pakhtunkhwa. Mapa została wygenerowana za pomocą bezpłatnego oprogramowania open-source qGIS (wersja 3.28) (GNU General Public License), opracowanego przez Open-Source Geospatial Foundation Project (http://qgis.org)

7.6. Discussion

At this instant, more than 100 countries including South Asia, Africa, America, Australia, the Caribbean, Pacific Islands, and Mexico that are positioned in steamy and subtropical regions have been considered as dengue endemic countries [1]. In Asia, Pakistan is measured as a dengue-endemic country after facing certain outbreaks [19]. In 1994 the earliest case of dengue fever appeared in Karachi but frequent outbreaks started after 2000 [20]. In KPK enduring epidemics of dengue have been recorded in 2013 in different regions including Kohat. Studies on dengue fever have been conducted in multiple cities of Pakistan including KPK, but it's for the first time in Kohat. This study describes the dengue fever epidemiology, risk factors, seasonal association and medical distinctiveness to analyze the dynamics of dengue fever in the district of Kohat. Kohat is situated on the route of hilly series; mountains and hills enclosed most of its fraction and the network of 3 dams and a small river deliver water into all areas by open canals which grant adequate establishment for mosquito breeding [21]. Hilly areas favor the activities of DENV vectors like Ae. Albopictus during monsoon season in dengue-endemic countries is reported in KPK, Pakistan as well [22]. Climatic factors like hot temperatures, heavy rainfall, and increased population have played a key role in the dengue fever expansion across the country.

The outcome of this study specified that 453 patients were diagnosed with dengue infection in a figure of 1742 subjects. The frequency of positive cases was restricted to 5 regions Lachi, Hangu, Shakardara, College town, and Kaghazai. The highest incidence was found among Lachi and Shakardara members as according to the study of S. Khan et al the water storage tanks are highly contaminated with Lachi and Shakardara [23] which are the most attractive sites for vector breeding. These are high cultivation territories and have open water channels and ponds. Meanwhile in the summer severe high rainfall; hot temperatures, and open stagnant water favor vector breeding to DENV spreading and establishment of infection [23].

In our study, we saw that males were more affected with a frequency of 62.91% while the rest were females that appeared to be less susceptible to infection, similar findings are already present in the literature [8, 24]. In a study, the percentage of males was 64% and females were 36% while in another study, the positive male was 89%. However, males are more susceptible to dengue infection as they are dependable for outer activities while female is less affected as they are restricted to the home. We also saw that rural areas are more affected by dengue infection in contrast to urban areas as rural regions are used for agriculture and cultivation and for that they need water reservoirs and supply which are usually uncovered and provide a favorable environment for DENV vector breeding, these results are in contrast with the study of Hayes et al. who reported urban areas are more susceptible as compared to rural areas [25].

Furthermore, we segregate results data into different age groups, the prevalence rate varies among different age groups, and the highest (38.18%) prevalence rate was observed in the 20–30 age group, these findings are similar to the previous studies [26]. The 10–20 age group showed the second highest prevalence (29.58%). This population is highly susceptible to dengue infection as this group people belong to school children, college students and employees mostly live outside of their homes. The age group 5–10 appeared less susceptible to infection as they lived mostly inside the home and they are safe up to some extent.

The positive cases started appearing in July, the prevalence rate was low in July and August though suddenly increased in October (36.20%) and September (28.25%) due to rainfall that favors the propagation of dengue vectors (Aedes). Similar results are present in the literature [8]. The positive patients were mostly illiterate; 45% were under Matric. Month-wise distribution of dengue infection revealed that vector-borne diseases like dengue are strongly influenced by seasonal variations and metrological factors. Additionally, open-source water supply was identified as a risk factor for dengue infection. Considerably all the patients got DF in the summer season and only a few were recorded as positive in winter (November). In our study post-monsoon season, temperature and rainfall appeared to have more impact on the transmission of infection. According to a recent study, a temperature of more than 25°C greatly favors mosquito breeding, increasing 1°C temperature to 26°C causes the possibility of dengue transmission chances up to 1.95 times [27, 28]. In our study, (68.33%) of patients were illiterate, as they were not equipped with knowledge of prevention measures for dengue infection, this analysis shows similarity with Khanani et al, who reported illiteracy and unawareness also played an important role in the prevalence of dengue fever [29].

In the current study, high-grade fever and shivering appeared to be the main symptom observed in all patients, these observations are similar to the findings of [30] clinical signs and symptoms of suspected patients play an important role in the diagnosis and treatment of DF [31, 32]. Presently there is no antiviral drug available for dengue infection in our country; the physicians prescribe only symptomatic treatment. Controlling DENV vectors and rapid diagnosis at early stages is the precise way to reduce infection rates [33]. According to our survey in Kohat, dengue infection is diagnosed in only two institutes DHQ and CMH. The diagnostic kits should be available in endemic areas, especially during August, September, and October for the early diagnosis to control the infection timely [34]. Kohat is a populated district with poor health care settings with no dynamic inspection organization of DENV and its vectors and exclusively counting on the inactive supervision system is not enough for the whole district.

7.7. Conclusion and Suggestions

Overwhelmingly this study represents the dengue endemic areas in Kohat which are Lachi, Shakardara, and Hangu which are highly cultivated areas comprised of greenery, and open water channels that facilitate mosquito breeding and other activities. Prevention is the only way to control DENV due to the lack of proper antiviral treatment. Control strategies are required to control vector behavior, water channels, and other water reservoirs need to be covered, and the removal of dull stagnant water on priority. Furthermore, rapid diagnostic facilities need to be developed in healthcare organizations, especially in endemic areas, and the identification of serotypes as well. Seminars and lectures need to be arranged in endemic areas for public awareness. Additionally, researchers need to improve the vaccination strategies with high efficacy.

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THE EPIDEMIOLOGICAL STUDIES OF DENGUE FEVER IN KOHAT

Abstract

Dengue fever, which is emerging as a major public health problem, affects 50–100 million people annually with particular reference to developing countries. Several regionsin Pakistan have suffered severe outbreaks over the past 15 years; however, the incidence of the disease in several cities including the southern parts of the Khyber Pakhtunkhwa province is under-reported. To find out endemic regions in district Kohat, this study was conducted in two major hospitals of the region including the district headquarters hospital (DHQ) and Combined Military Hospital (CMH) from July 2018 to November 2019. The IPD patients were directly interviewed while in the case of OPD patients, blood samples were analyzed for IgG or IgM antibodies using ELISA. We examined 1742 individuals experiencing dengue-like symptoms for the presence of IgM or IgG levels. Among these, 453 (26%) individuals were positive for either IgG or IgM. The frequency of infection was higher in males 70.42% (319) compared to females 29.58% (134). Most of the cases (46%) were from the Lachi area, followed by Hangu and Kohat cities. The incidence rates varied with season with 64% of cases reported in the post-monsoon season. This study identified 3 different dengue endemic areas in district Kohat which need a dynamic surveillance system for the control of dengue fever to minimize its risk. Healthcare officials need to establish rapid diagnostic facilities in endemic areas accompanied by vector control strategies.

Keywords: Dengue, Prevalence, Kohat, DHQ, CMH, Climate, Monsoon