Epidemiological features of dengue fever in a four-year hospital-based study in Faisalabad, Pakistan

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ABSTRACT

Background: Pakistan, being a subtropical country, is a source of various vector-borne diseases including dengue, malaria, chikungunya, Congo fever etc. Dengue fever has recently become a rapidly emerging infection with abundant morbidity and mortality in this region.

Objectives: Current study undertakes to determine the epidemiological features of dengue patients reported to a tertiary care hospital in Faisalabad, Pakistan.

Patients and methods: This four-year (2014-2017) retrospective cross-sectional study was carried out at Allied Hospital Faisalabad. Data of the hospitalized dengue patients was studied for demographic details, travel history to the dengue endemic areas, diagnostic information and impact of co-morbidities on the severity of the disease. Furthermore, usefulness of Google Earth® software was explored for tracking of dengue cases.

Results: A total of 155 confirmed dengue cases were reported including 27% females and 73% males with a mean age of 29.5 years. Among them, 93.5% were reported with dengue fever, while 6.5% were reported with dengue hemorrhagic fever. Three different dengue serotypes were detected in the patients including DENV-1 (7.7%), DENV-2 (17.9%) and DENV-3 (74.4%). Malarial co-infection was detected in 13% of the dengue cases among which 10.5% developed severe dengue fever. Map of the city built in Google Earth® showed that 76% patients were reported from highly populated Central region of the city. Total 28 cases reported with dengue infection having travel history to Lahore, Rawalpindi, Karachi and Islamabad. Additionally, 04 international travelers with dengue infection coming from Saudi Arabia, Malaysia and Rwanda were also reported to Faisalabad.

Conclusions: Circulation of various dengue serotypes in Faisalabad population require urgent attention of the public health authorities and policy makers to deal with the escalating problem of dengue fever in Pakistan.

Keywords:
Dengue, serotypes, dengue-hemorrhagic fever, Pakistan.

INTRODUCTION

Arboviral diseases including dengue fever, chikungunya, Zika fever etc., remained a major public health challenge in both industrialized and developing countries around the globe.[1,2] The diseases transmitted by mosquitoes, fleas and ticks have been more than tripled in US from 2004 to 2016. Furthermore, nine new germs transmitted by mosquitoes (including Zika virus) and ticks have been discovered since 2004.[3] Especially, dengue and other mosquito-borne infections have become very common and thus considered as most important infections in terms of human morbidity and mortality.[1,2,4] According to recent estimates, about 390 million dengue infections are reported annually from more than 100 countries, among which about 300 represent subclinical/asymptomatic while approximately 96 million represent symptomatic infections.[5]

Dengue is caused by an enveloped positive sense ssRNA virus known as dengue virus (DENV) belonging to the subgroup of genus *Flavivirus* of family *Flaviviridae*. DENV has four closely related but serologic distinct serotypes: DENV-1, DVEN-2, DENV-3 and DENV-4. The virus is principally transmitted to the humans by *Aedes aegypti* (*A. aegypti*) and to a lesser extent by *A. albopictus* mosquitoes.[4] DENV infections may present as asymptomatic and undifferentiated fever to mild dengue fever (DF) to severe dengue manifestations as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).[6,7] Dengue fever usually produce a self-limiting flu-like illness characterized by sudden onset of fever, headache, fatigue, nausea, vomiting, rash and myalgia in humans.[8]

Previously dengue-free countries like Pakistan, Saudi Arabia, Yemen, Sudan, and Madagascar are now facing outbreaks of dengue infection. Because of subtropical location of Pakistan, it is the hot spot for many vector borne diseases like dengue, malaria, leishmaniosis, West Nile virus and Crimean-Congo hemorrhagic fever.[9] Dengue vector was present in this zone even before the division of Indian subcontinent. First epidemic of DF in Pakistan due to dengue serotypes-1 and 2 was reported in 1994 affecting thousands of people. Afterwards several dengue virus outbreaks have been reported in Pakistan in the last 30 years.[9] Pakistan was hit by devastating floods in 2010 and 2011 which not only destroyed properties and claimed several precious lives but also produced favorable breeding conditions for the mosquitoes. Therefore, worst strike of dengue fever infecting more than 20,000 cases and causing 300 deaths was reported in 2011.[10-12] According to experts, these figures still reflect under reporting. Maximum numbers of cases were reported from Lahore followed by Faisalabad, Rawalpindi and Sargodha during this outbreak.[10-12]

Over the past two decades there is a tremendous change in epidemiology of dengue in South East Asia. Yet, the data regarding overall trend of dengue infection, especially in this part of the world, remains scarce. Faisalabad city is the industrial hub and the third most populous city of Pakistan after Karachi and Lahore having a population of about 3.204 million (population census 2017).[13] This study undertakes to determine the demographic and diagnostic characteristics of dengue patients reported to tertiary care hospital in Faisalabad. Furthermore, for the first time utilization of Google Earth® software to pinpoint the hotspots for dengue infection in Faisalabad is reported.

PATIENTS AND METHODS

This retrospective cross-sectional study was carried out at Allied Hospital Faisalabad, a tertiary care teaching hospital attached to Faisalabad Medical University, Pakistan. Allied Hospital Faisalabad is about 1150 bedded hospital designated as specialized Centre for the treatment of dengue patients, therefore all the suspected/positive cases around the district were referred to the hospital for management. Data of the dengue patients reported during 2014-2017 was studied for demographic details, travel history to the dengue endemic areas, co-morbidities and diagnostic information. Dengue cases were divided into dengue fever (DF) and dengue hemorrhagic fever (DHF) on the

basis of World Health Organization (WHO) classification and case definition.[4] A patient was designated as a confirmed case of dengue fever based upon clinical and serological evidence. Immunoassay (ELISA) was used for the diagnosis of the patients. A patient reported within 2-4 days of fever was tested for NS1 antigen and patient index >0.482 was taken as positive. Furthermore, NS1 positive serum samples were used for dengue serotype analysis through PCR. All the suspected patients were also tested for anti-dengue IgM and IgG antibodies. A patient index >0.888 and >0.682 was taken as positive for IgM and IgG, respectively. Patients having index value less than the cut-off value were excluded from the study. Patient complete blood picture was studied for four parameters: hematocrit, hemoglobin, leukocyte count and platelets count. Hematocrit (HCT) greater than 48% was regarded as increased. Hemoglobin (Hb) concentration lower than 11.2g/L was taken as low in children while, its concentration lower than 12 and 13g/L in females and males respectively were taken as decreased. Thrombocytes lower than 150,000 cells/mm^3 was taken as thrombocytopenia. Leukocytes count lower than 4.5 cells/mm^3 was taken as low.

Google Earth® is a computer software developed by Google Inc., which shows 3D representation of the earth based upon satellite imagery. The software is made available to the users free of cost by the developers. The software is easy to use and can be installed on various operating systems. Current study additionally explored the usefulness of Google Earth® software in low income settings, like Pakistan, as an alternate tool to Geographic Information System (GIS) by building map of Faisalabad city for dengue epidemiology. For this purpose, Google Earth® Pro (version 7.3.1.4507) was used. Faisalabad city was located by using search option in the Google Earth® software. A list of different towns/societies within the city was obtained upon which a map was built. Orthogonal tool (available in the "Add" pull down menu) was used to draw the map of the whole city. Each area within the city was searched separately and a boundary was drawn using the tool. Placemarks were used to indicate areas from where dengue cases were reported, where number on each placemark represent frequency of dengue cases from that specific area. Customized placemarks were also prepared in Microsoft PowerPoint as per requirement, which were imported to Google Earth® for use. A combined map was built by pooling data of the patients reported during 2014 to 2017 from different areas using placemarks.

Data was entered and analyzed in SPSS version 22. The p-value was calculated by χ^2-test for categorical variables, while t-test was used for quantitative variables. A p-value less than 0.05 was considered as significant.

RESULTS

A total of 155 confirmed dengue cases were reported to Allied Hospital Faisalabad during the study period including 42 (27.1%) females and 113 (72.9%) males. Mean age of the patients was 29.5 years (range, 2 to 80 years), including 18 (11.6%) children (less than16 years of age) and 133 (88.4%) adults, among whom 75 (56.4%) were young adults (17-30 years old). Maximum number (51.6%) of patients were observed in the age group of 16-30 years (Figure 1). A slight decline (p-value>0.05) in the median age of patients from 27 to 25 years was noted over the past four years study period (2014 to 2017).

Among total 155 dengue patients, 145 (93.5%) were reported with dengue fever while 10 (6.5%) were reported with dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS). The mean age of the DHF patients was lower (26 years), although not significantly (p-value=0.819), as compared to DF patients (29.8 years). Similarly, no significant difference was noted for gender and laboratory findings among DF and DHF cases. However, a significant difference was noted for the value of hematocrit, which was significantly higher (p-value = 0.015) in DHF (43.1%) as compared to DF patients (39.6%). Table 1 shows comparison of demographics and laboratory finds according to severity of dengue fever.

Different diagnostic tests including immunological and molecular tests were performed for the confirmation of dengue virus infection. Total 57 (36.8%) cases were NS1 positive while 39 (68.2%) were PCR positive as well. Three different dengue serotypes were detected in the patients including DENV-1 (7.7%), DENV-2 (17.9%) and DENV-3 (74.4%), while
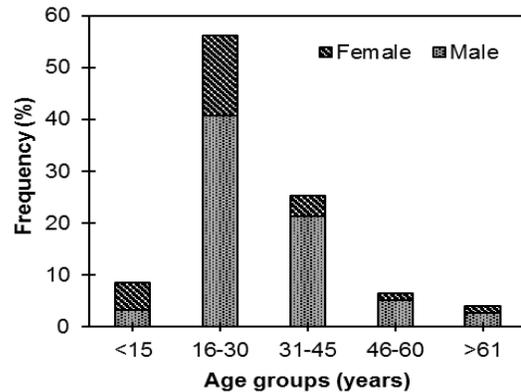

Figure 1. Gender-wise distribution of dengue cases into different age groups.

DENV4 was +not detected in any patient. Data was also collected about the presence of malarial co-infection in the dengue patients. Total 19 (13.1%) out of 145 dengue cases were tested positive for malarial parasite. Among them, 2 (10.5%) patients positive for *Plasmodium falciparum* (*P. falciparum*) developed DHF, while 1 (5.2%) of them went into shock (DSS). Table 2 summarizes the laboratory tests performed for the diagnosis of dengue infection.

Google Earth® Pro was used to construct map of the Faisalabad city to pinpoint the location of each dengue case reported from different areas within the city. Possible hotspots were pointed out by building combined map highlighting areas from where cases were reported in four consecutive years. Total 118 (76.2%) cases were reported within the city having no travel history to dengue endemic areas. Most of the cases were reported from central more populated urban region of the city. Although mostly 1 to 2 cases were reported from most of the areas however up to 18 cases were reported from certain parts. Figure 2 shows map of Faisalabad city showing number of cases reported from different areas within the city.

Table 1. Characteristics of dengue patients reported to Faisalabad, Pakistan

Characteristics	DF (%)	DHF (%)	N	p-value*
Demographics				
Age [mean]	29.8	26	148	0.819
Gender				
Male	104 (71.7)	09 (90)	113	0.209
Female	41 (28.3)	01 (10)	42	
Laboratory findings				
Low Hb	37 (25.5)	03 (30)	135	0.894
Thrombocytopenia at presentation	143 (98.6)	10 (100)	153	0.709
Leukopenia	127 (87.5)	10 (100)	155	0.495
HCT level on admission [mean]	39.6	43.1	131	0.015

*p-value was calculated by χ^2-test for categorical variables, while t-test was used for quantitative variables. A p-value less than 0.05 was considered as significant.

Table 2. Laboratory findings of dengue patients reported to Faisalabad, Pakistan

Laboratory Investigations	N (%)
Immunodiagnosis (N=155)	
NS1	57 (36.8)
IgM	105 (67.7)
IgG	49 (31.6)
IgM + IgG	42 (27.1)
Molecular diagnosis (N=39)	
DENV1	03 (7.7)
DENV2	07 (17.9)
DENV3	29 (74.4)
Co-infection (N=145)	
Malarial parasite (*P. falciparum* and *P. vivax*)	19 (13.1)

Total 28 cases reported with dengue infection having history of travel to major dengue endemic cities of Pakistan, including Lahore (60.7%), Rawalpindi (10.7%), Karachi (14.3%) and federal capital Islamabad (14.3%). Additionally, 4 international travelers reported visits to dengue endemic countries, including 2 (50%) cases from Malaysia, 01 (25%) from Saudi Arabia and 01 from Rwanda.

DISCUSSION

Dengue infection was more frequently reported in males (72.9%) as compared to females (27.1%). It is noteworthy that according to recent population census (2017) the male to female ratio in Pakistan is almost similar (i.e. 1.05:1).[13] Therefore, this gender difference could be due to other reasons like exposure and cultural reasons, for instance the females spend most of their time indoor as compared to male counterparts.[7,10] Overall a similar trend was seen among different age groups (Figure 1) with the exception of ≤15 years old children among which the frequency of female patients were higher (8%) as compared to males (5%).

Data about the DENV serotype analysis showed that three different serotypes were circulating in the city including DENV-1, DENV-2 and DENV-3. It is noteworthy that DENV-1 was only reported in the patients having travel history to the dengue endemic cities (Lahore, Karachi) of Pakistan. Many previous reports confirmed co-circulation of all different dengue virus serotypes in these cities.[14,15] Faisalabad being the industrial hub of Pakistan is an attractive place for business community who could contribute significantly in importing new DENV serotypes to the local population. In 2011 outbreak, the authors had reported three different serotypes including DENV-2, DENV-3 and DENV-4 circulating in the local population. Therefore, it may not be far away when all four serotypes will be circulating in the city, which is a

matter of serious concern requiring urgent remedial action by the public health authorities. It is well known that primary dengue infections are generally asymptomatic and confer lifelong immunity against the respective serotype however, secondary infection with different DENV serotype might result in more severe form of dengue infection (i.e. DHF/DSS), depending upon the time interval between both primary and secondary infections. [4,16,17]

Use of geographic information system (GIS) has been suggested as an important tool for the control of vector borne diseases.[18] However, in resource-limited settings like Pakistan, basic information is not only lacking but also infrastructure (i.e. trained technical personnel, modern technology and GIS software) is absent, therefore the use of Google Earth® was explored by the researchers to overcome these hurdles. Recently, Google Earth® was used in public health for the control of non-communicable diseases, and improvement of sanitary conditions in India.[19] Likewise, current study utilized Google Earth® software to track dengue cases and identify hotspots for the dengue infections in Faisalabad (Figure 2). Most of the cases were reported from heavily populated urban region of the city. Additionally, significantly higher number of patients were reported from some of the regions in the city, which coincide with the old market places and industrial warehouses in the city. Such information could be utilized by public health and other government institutes not only for the control of dengue vector in those areas but also for the improvement of health and socioeconomic conditions of the masses in general.

International travel contribute significantly in the spread of infectious diseases around the globe. Many studies have demonstrated importation of dengue virus from dengue endemic areas through international travelers.[20,21] Similarly, four international travelers were reported with dengue infection in Faisalabad.
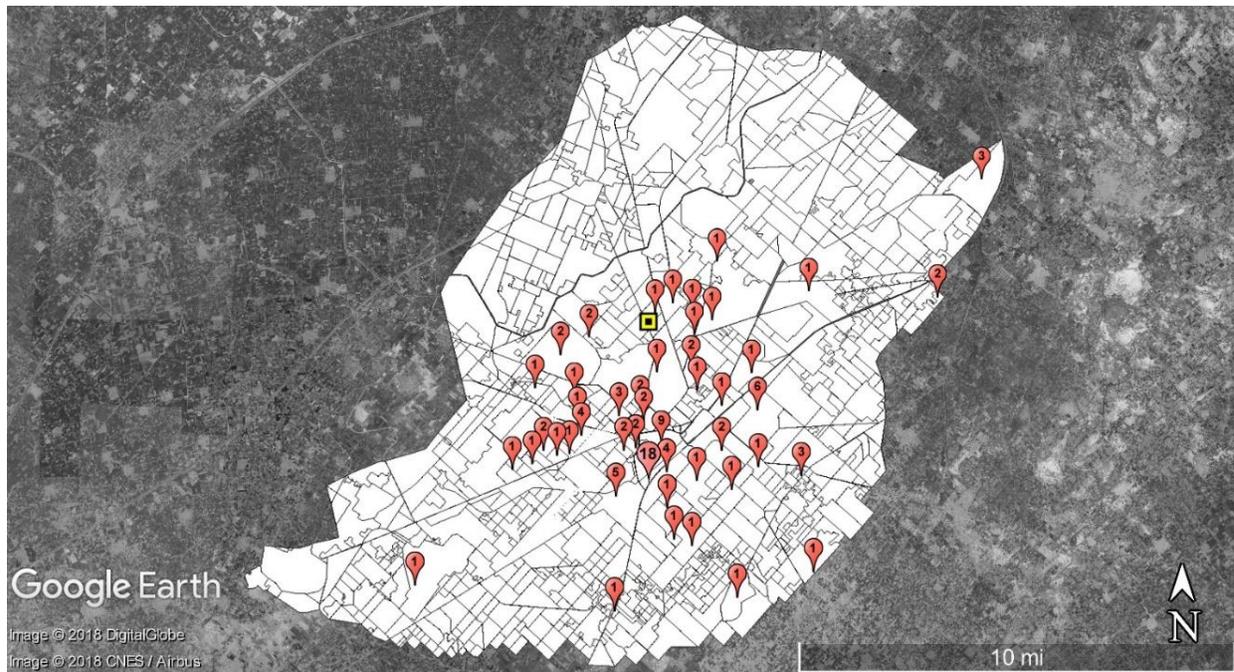
Figure 2. Map of Faisalabad city constructed in Google Earth® software. Boundary lines represent different residential/non-residential blocks within the city. Red placemarks represent location of dengue case(s) while number on each placemark represent number of cases from each of these locations. Study site is shown here by yellow placemark.

Additionally, influx of large number of local travelers of from major dengue endemic cities of Pakistan might have played a significant role in the introduction various dengue serotypes to Faisalabad. Recently, mobile phone based mobility data of ~40 million people from dengue endemic areas in Pakistan to Faisalabad showed that international/local travelers played significant role in the spread of dengue infection to Faisalabad.[22]

Concurrent infections are thought to be risk factor for the development of severe dengue fever (DHF/DSS). However, negligible data is available on this topic both at international and especially at national level. In this study, total 19 cases of dengue fever with co-infection of malarial parasite were detected. However, only two cases with *P. falciparum* infection were reported with severe dengue fever and severe internal bleeding (HCT<30%). One of them was an 11 years old male and the second was a 12 years old female. Previous studies demonstrated co-infection with malarial parasite as a risk factor for the development of severe form of dengue fever and responsible for more sever thrombocytopenia and anemia.[23] However, conflicting data is available on this topic and more comprehensive studies are needed to reach any logical conclusion.

CONCLUSIONS

Circulation of various dengue serotypes in Faisalabad population require urgent attention of the public health authorities and policy makers to deal with the menace of dengue fever. Hotspots utilizing existing software pointed out in this study could be utilized for effective vector control measures. Furthermore, effective screening of the international travelers for infectious diseases could be helpful in minimizing the importation of deadly and new diseases to Pakistan.

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