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Dengue Outbreak in Peshawar: Clinical Features and Laboratory Markers of Dengue Virus Infection

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ABSTRACT

Background: Dengue is a tropical disease caused by dengue virus (DENV). It is one of the most significant arthropod-borne viral infection.
Objectives: The aim of the current study was to characterize epidemiological, clinical features and laboratory markers of dengue infection during the recent epidemic in Peshawar, KP.
Methods: During the prospective hospital-based observational study, 2000 dengue suspected cases were serologically examined at Lady Reading Hospital (LRH) Peshawar. Dengue NS1 antigen and Dengue IgG and IgM antibody ELISA tests were conducted for the confirmation of dengue infection. Data regarding the clinical features, platelet count and liver function tests were also recorded for the dengue positive patients.
Results: Out of total samples 415 (21%) cases including 309 (74%) male and 106 (25%) female were detected positive for the dengue infection. In the dengue positive patients, the highest prevalence was observed in the age group of 21–40 years with 160 (38%) followed by the age group of 1–20 years with 89 (21%) patients. Fever was recorded in 100% of the dengue patients followed by a headache and fatigue in 73% and liver abnormality observed in 70% of the cases. During laboratory examinations IgM antibody was detected in 180 cases, followed by IgG antibody in 87, NS1 antigen in 43, NS1 antigen along with IgG and IgM antibodies in 41 dengue positive cases. Another combine detection of NS1 antigen with IgM antibody, NS1 antigen with IgG antibody and both IgG and IgM antibodies was observed in 21, 21, 22 dengue cases respectively.
Conclusion: It was concluded that the dengue infection can be early diagnosed on the basis of described clinical features and with the detection of dengue-specific NS1 antigen along with antibodies such as IgG and IgM.

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Introduction

Dengue is a tropical infection which is caused by the dengue virus. DENV is a positive single-stranded RNA virus which belongs to the family Flaviviridae and genus Flavivirus [1]. DENV is a vector dependent viral infection which poses a major health threat globally. An estimated 280–530 million cases of dengue infections were reported in 2010 globally and clinically apparent cases were about 70–140 million [2,3]. According to the report of the World Health Organization (WHO), there is a tremendous increase in the incidence rate of the dengue infection and pose a global health catastrophe [4]. The infection can be the outcome of the transmission of any of the 4 dengue serotypes. The infection can be asymptomatic or can also give rise to an indistinguishable fever which can be associated with other health abnormalities including Dengue hemorrhagic fever (DHF), Dengue fever (DF) or Dengue shock syndrome (DSS) [1].

The mortality rate is less than 1% when clinically administered with proper care [1] and usually stands at 1–5% without sufficient clinical management [5]. In certain cases, the mortality rate stands at 26% with a severe form of the disease [5,6]. The common mosquito vectors for the transmission of the infection are *Aedes aegypti* and *Aedes albopictus* [7]. The disease has penetrated its roots into the foundation of many nations and is now endemic in more than 100 countries including Africa, the Eastern Mediter-

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ranean, America, Western Pacific and South-east Asia [8]. Dengue infection is very common among travelers from the regions situated in the tropics and subtropics [9]. The risk of the infection in the travelers usually depends on the exposure to dengue vectors and the local prevalence of the infection. In the risk classification for dengue, the areas are classified based on the evidence of a particular number of cases in at least 3 years of the previous decade. The areas are either classified as frequent/continuous risk with evidence of more than 10 cases or classified as a sporadic/uncertain risk with at least one dengue case [3].

Primary exposure to the infection leads to the development of the lifelong immunity against the particular serotype. While in case of the secondary infection there is usually the risk of development of a more severe form of the disease i.e. DHF and DSS [10]. In terms of the pathophysiology associated with the disease in later phenomena, it could be efficiently explained with the theory of Antibody-Dependent Enhancement (ADE) [11], which has proven to be the worst obstacle in the route heading toward the development of effective vaccines against the 4 serotypes of the dengue virus [12]. However, it is determined that with the postsecondary exposure the risk for the development of the severe disease during the tertiary and quaternary infections significantly reduces with either of the two remaining serotypes as a consequence due to the development of the protective immunity [13].

The DENV has been endemic for many years in Pakistan because of the temperate climate of the country [14,15]. Multiple dengue outbreaks have been reported since 2006 every year along with the circulation of the multiple serotypes [16]. Such outbreaks are primarily because of the vast agriculture land, rich fauna, water reservoirs for power generation, open irrigation channels and floods because of heavy rainfall provides very suitable sites for the breeding of the mosquitoes. The mosquito vector activities vary greatly in accordance with the seasonal ramification in various geographical areas of the country. However, the incidences of the infection increase after rainy seasons [1].

The first case on dengue infection in Pakistan was reported from Punjab in 1982 and since about 8–10 outbreaks of the disease have been reported from the country [15,17–21]. A major epidemic of the infection was reported from Punjab in 2011 with an estimated number of reported cases to be more than 50,000 from Lahore only till the end of November 2011 [22]. In 2013 another huge outbreak of the infection was reported from Khyber Pakhtunkhwa (KP) province, Pakistan. According to the report of WHO in 2013 the estimated number of the cases were 8546 which included 33 deaths in District Swat. The prevalent serotypes that caused the outbreak were serotypes 1, 2 and 3 [23]. Another study reported the number of cases in Swat to be around 6000, out of which 69% of patients were male [24]. Random cases of the infection were also reported from other 3 provinces of the country. During 2013 in Punjab, about 2165 cases of the disease were reported by various health departments and from the total cases, 50% were alone reported from Rawalpindi [25].

Materials and methods

The current study was conducted in Lady Reading Hospital (LRH) Peshawar. The study was approved by the ethical committee of Bacha Khan University, Charsadda, KP and the medical superintendent (MS) of LRH. The study was conducted from June 2017 to May 2018.

Sample size and inclusion criteria

In the current study, 2000 patients were suspected of the dengue infection. Data regarding the clinical analysis, signs, and symptoms were recorded from the dengue patients. The inclusion criteria included the diagnosis of dengue fever having positive NS1 antigen, IgG and IgM antibodies. All the patients were thoroughly examined for fever, Haematemesis, skin rashes, enlarged liver, vomiting, abdominal pain, nose, and gum bleeding. The laboratory analysis performed were liver function tests, platelets count, NS1 antigen, IgM and IgG antibodies.

Table 1
Gender wise distribution of dengue infection.

Gender	Positive	Negative	Total
Male	309	691	1000
Female	106	894	1000
	415	1585	2000

Laboratory procedure

Blood was taken from the patients and was centrifuged at 3000 rpm for 10 min. The sera were transferred to fresh microfuge tubes. Serum after extraction was stored at −80 °C until further use. Each sample was analyzed for the detection of the NS1 antigen and both antibodies IgM and IgG by using dengue NS1 antigen capture ELISA and dengue IgG, IgM capture ELISA according to the manufacturer protocol. From each dengue positive patients data was recorded for common fever, abdominal pain, vomiting, abnormally enlarged liver, nose bleeding, gum bleeding, skin rashes, headache, fatigue, and hematemesis (blood vomiting).

Definitions of primary and secondary dengue infections

The infection was classified as primary and secondary based on the serological patterns of the infected patients. NS1 antigen holds a promise for the rapid detection of the dengue infection and is thus considered as an important marker for the determination of primary infection. During NS1 positive, IgG and IgM negative case the infection will be defined as primary infection. It can also be during the positive IgM and negative IgG cases. In the case of negative IgM and positive IgG case, the infection will be defined as a secondary infection.

Statistical analysis

The data analysis was done with SPSS version 21 (IBM SPSS Statistics v21).

Results

A total of 2000 dengue suspected individuals were examined, out of which 415 (21%) patients were infected with dengue virus. In the dengue positive patients, 309 (74%) were male and 106 (25%) were female as shown in Table 1.

Evaluation of platelet count

The platelet count was determined in all the dengue positive patients. In 60 cases (14%) the platelet count was <25,000 followed by 83 (20%) cases with 25,000–49,000, 94 (23%) cases with 50,000–74,000, 68 (16%) cases with 75,000–100,000 and 110 (26%) cases were having platelet count 100,000 as shown in Table 2.

Prevalence in various age groups

In the current study, the patients were classified into various age groups. The relative prevalence was determined in the age groups. The highest prevalence was observed in the age group 21–40 years

Table 2
Platelet count.

Platelet count	<25,000	25,000–49,000	50,000–74,000	75,000–1,00,000	100,000
Male	45	62	76	36	90
Female	15	21	18	32	20
Total	60	83	94	68	110

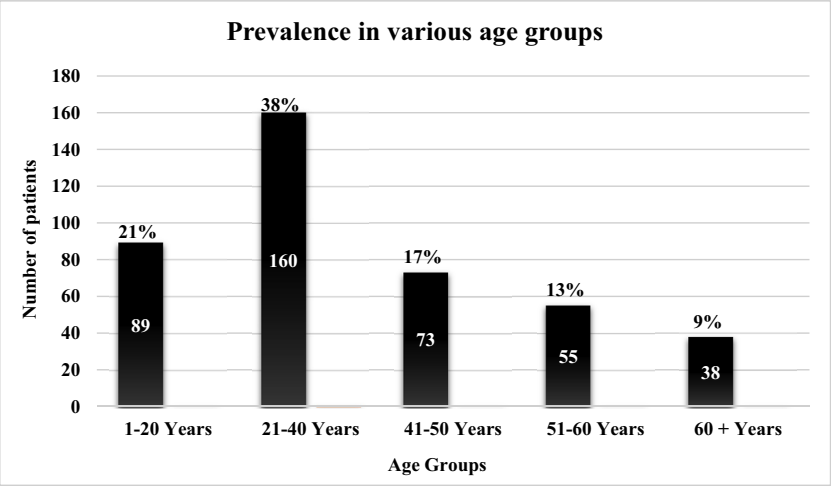

Fig. 1. Prevalence of dengue infection in various age groups.
In the above figure, the overall prevalence of dengue infection was determined in various age groups. Highest prevalence was observed in the age group of 21–40 years with 38% followed by age groups of 1 20 years and 41–50 years with 21% and 17% prevalence.

Table 3
Liver function tests.

Patients	Raised AST	Raised ALT	AST > ALT	Hyperbilirubinemia
415	91%	80%	+	7%

AST: Aspartate transaminase; ALT: Alanine transaminase

with 160 (38%) followed by the age group of 1–20 years with 89 (21%) patients. However, 73 (17%), 55 (13%), and 38 (9%) was the frequency observed in age groups of 41–50 years, 51–60 years and in 60 years and above age group respectively as shown in Fig. 1.

Clinical features of dengue-infected individuals

The common clinical features in dengue-infected patients were also determined. Fever was very prominent and was observed in almost all dengue patients. A headache and fatigue were observed in 73% of the patients followed by symptoms of the enlarged liver with the frequency of 70%. Other clinical features including nose bleeding, gum bleeding, hematemesis, abdominal pain, vomiting, and skin rashes were having a frequency of 60%, 54%, 52%, 50%, 43%, and 30% respectively as shown in Fig. 2.

Liver function abnormalities in dengue patients

The most common abnormalities were observed in the transaminase levels. Elevated AST levels were observed in the 91% of cases while high ALT levels were observed in 80% of the total cases. Out of total cases, the Hyperbilirubinemia was observed in 7% of the cases. The results are shown in Table 3. Elevated levels of AST, ALT and abnormality in Bilirubin levels has also been reported in a study from the studied area [26].

Detection of NS1 antigen, IgG, and IgM antibodies

The dengue-infected patients were examined for the presence of Dengue NS1 antigen and antibodies including IgG and IgM for the confirmation of cases. IgM antibody alone was more prevalent in the majority of the cases with the frequency of 124 male and 56 female patients. IgG antibody alone was second most prevalent with 67 male and 20 female patients cases. NS1 antigen was observed in 33 male and 10 female patients. The presence of NS1 antigen along with IgG antibody was observed in 18 male and 3 female cases, followed by NS1 antigen and IgM antibody presence in 16 male and 5 female cases. The frequency of the presence of NS1 antigen along with both antibodies IgG and IgM was 35 male and 6 female cases. Presence of both antibodies without NS1 antigen was observed in 16 male and 6 female cases as shown in Fig. 3.

Discussion

Dengue is one of the tropical diseases that is underreported during most of the epidemics while over reporting occurs at some hospitals. The lack of laboratory resources and the non-severe cases with non-specific clinical presentation greatly worsen the reporting situation of the disease. The dengue virus might have been endemic in Pakistan which remains unnoticed because of the inadequate surveillance and diagnostic facilities. According to an epidemiological report during the 1960s and 1980s significant proportions of the normal healthy people were found positive for haemagglutination inhibition antibodies specific for dengue in Rawalpindi, Peshawar and Karachi [14] and other parts of the Punjab [14,27,28]. In the current study, the burden of dengue infection during the recent epidemic in Peshawar was determined along with the clinical characteristics, and laboratory findings for dengue-specific NS1 antigen and IgG and IgM antibodies. During the study 2000 suspected dengue cases were examined in which 415 (21%) were detected positive.

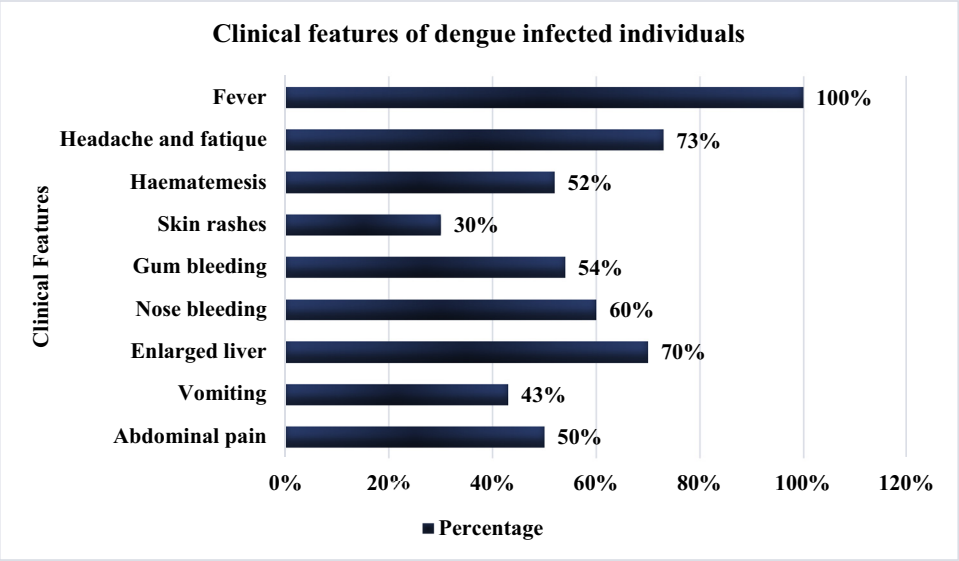

Fig. 2. Clinical features of dengue-infected individuals.
In the above figure, the clinical features of the dengue-infected patients were recorded. Fever was observed in 1005 of the cases followed by a headache and fatigue with 73%, enlarged liver with 70%. Frequency of other clinical symptoms was also recorded.

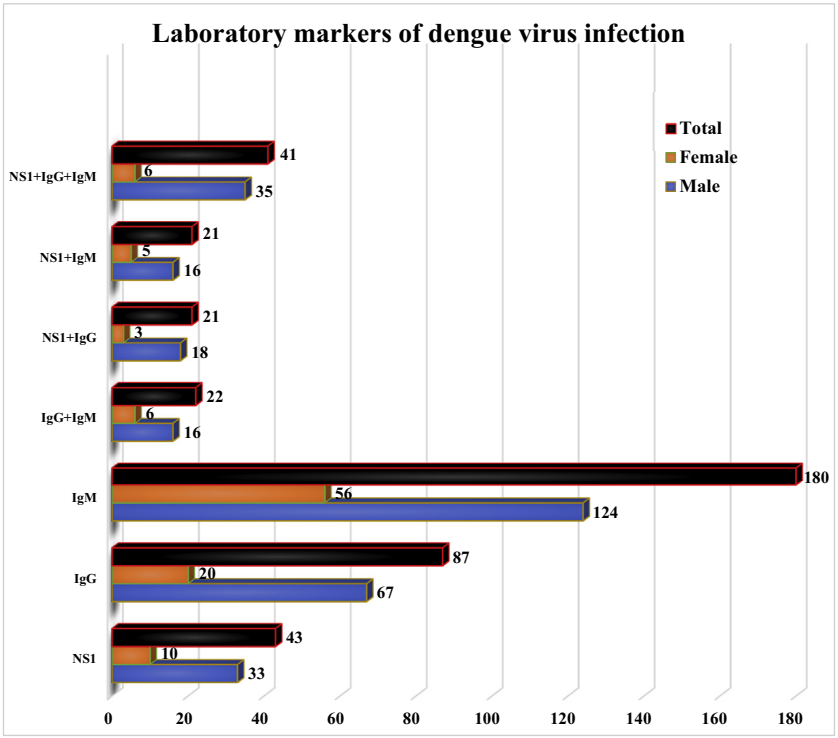

Fig. 3. Laboratory markers of dengue virus infection.
In the above figure, the frequency of laboratory markers associated with the detection of dengue infection was also determined. IgM antibody was observed in 180 cases followed by IgG antibody in a total of 87 cases. In some patients, both antibodies were also detected in some of the cases along with NS1 antigen.

In the studied population, fever was very prominent and was observed in almost all dengue patients followed by headache, fatigue, enlarged liver, nose bleeding, gum bleeding, hematemesis, abdominal pain, vomiting, and skin rashes. Several other studies have reported variable clinical complications of dengue fever with nil or scarce data regarding the early clinical manifestations. The results were in accordance with a study from Singapore, in which headache was observed in 80%, myalgia in 69%, abdominal pain in 11% and retro-orbital pain observed in 26% of the dengue patients [29]. In another study, the most common symptoms observed among the infected patients were fever with 100% frequency, followed by vomiting with 70%, abdominal pain with 50%, skin rashes with 19%, splenomegaly with 34% and gum bleeding with 3% [30].

In the current study, IgM antibody alone was more prevalent in the majority of the cases. IgG antibody alone was second most prevalent followed by NS1 antigen alone. The NS1:IgG and NS1:IgM were observed with same ratio in the studied population. However the NS1 antigen along with both antibodies (IgM, IgG) was observed with relatively high frequency as compared to NS1 antigen with either of the antibody. The results were in accordance with a study

in which 4121 male were examined for anti-dengue antibodies in which NS1 was prominent and observed in 3359 cases followed by IgG and IgM in 762 cases. In female NS1 was observed in 1171 cases followed by IgG and IgM in 708 cases [30].

Our data suggested the utmost need for proper monitoring of the clinical characteristics corresponding to laboratory findings during the course of infection. The patients predominantly having mild symptoms and treated as outpatients must be informed about the clinical warning signs in association with the outpatient monitoring during the infection. The vector control, garbage and sewage collection and the social factors of the health system are the key factors that play a significant role in the dengue epidemics. Educational and awareness campaigns along with the local epidemiological settings will serve as a dynamic dengue vector control strategy and will result in the eradication of the disease.

Conclusion

The epidemiological studies and determining the laboratory and clinical characteristics are very significant in the evaluation of the current diagnostic and therapeutic strategies developed for the prevention of dengue pandemics. Extensive research is of utmost importance along with the educational programs to bring awareness among the public about the possible risk factors and about the preventive measures for the infection.

Authors contribution

MH participated in the conception, design, and drafting of the manuscript, HJ participated in the collection of data, SF participated in the analysis of data, NA and MK participated in the interpretation of the data and FU was involved in the design of the study. All authors read and approved the final manuscript.

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Ethical approval

The study was approved by the ethical committee of Bacha Khan University, Charsadda, KP. Permission was also granted by the medical board of Lady Reading Hospital (LRH), Peshawar, KP.

Competing interest

None declared.

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