RESEARCH PAPER

Assessment of epidemiological and hematological parameters of dengue virus infection in District Mardan, Khyber Pakhtunkhwa, Pakistan

Shah Faisal[1], Hasnain Jan[2], Muhammad Haroon[2*], Yasir Khaqan Habib[1], Farhan Ahmad[1], Ajmal Khan[1]

[1]*Department of Biotechnology, Bacha Khan University, Charsadda, KP, Pakistan*

[2]*Department of Biotechnology, Quaid-i-Azam University, Islamabad, Pakistan*

Abstract

Dengue is a tropical disease caused by dengue virus (DENV). It is one of the most significant arthropod-borne viral infection. Dengue fever has been reported from various parts of Pakistan. However, there is still a scarcity of data on the prevalence and incidence of dengue. The aim of the present study was to determine the seroprevalence and hematological changes in dengue virus-positive patients in district Mardan, Khyber Pakhtunkhwa (KP). In the current study, 1000 suspected individuals including 632 male and 368 female samples were collected from different areas of district Mardan. Out of 632 male individual samples, 88 were positive while 46 out of 368 female samples were found positive for dengue infection. The primary and secondary infection was determined through IgM (M=49, F=21) and IgG (M=19, F=15) ICT, and through NS1 (M=20, F=10) ICT strips. Dengue fever also causes hematological changes in infected patients. The complete blood count (CBC) was determined for patients which undergoes hypo-volumatic shock condition. In the infected individuals low TLC, low PLT, high Hb and high HCT values were observed. In the current study, the frequency of infection was higher in male as compared to female. It was also 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.

* **Corresponding Author:** Muhammad Haroon ✉ haroon_ibgian@yahoo.com

Introduction

Dengue is a severe emerging disease, which is caused by a virus known as dengue virus (DENV). It is usually transmitted to humans by different types of mosquito vectors including *Aedes aegypti* and *A. albopictus*. The virus completes its life cycle in human and mosquitoes (Ali, Ahmad *et al.* 2016). DENV is a single-stranded RNA virus belongs to the genus *Flaviviruses* consisting of 3 structural (capsid, membrane, and envelope) and 7 non-structural (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) proteins. Mainly dengue infection is caused by one of the four different serotypes: DV-1, DV-2, DV-3 and DV-4 (Cardier, Mariño *et al.* 2005). Dengue fever is increasing with an alarming rate and annually 50-100 million cases reported worldwide and among these, the identified cases of dengue hemorrhagic fever are approximately 250,000-500,000 (Chen, Yang *et al.* 2007). Dengue can be classified into an acute febrile illness called dengue fever (DF), DF with a bleeding tendency (DF w/B) and Dengue hemorrhagic fever (DHF). DF w/B and DHF are life-threatening stages of dengue fever (Diamond and Pierson 2015).

Epidemic dengue is the main health threat in tropical monsoon and equatorial zones where *A. aegypti* is widely spread and considered as a leading cause of hospitalization and deaths in children. In 2003 dengue epidemics were reported in Bangladesh, India, Indonesia, Maldives, Myanmar, Sri Lanka, and Thailand. In Pakistan, Dengue fever was reported in 1991 while in 2005, DV-3 epidemic with DHF was reported for the first time and then it was expanded to major cities of Khyber Pakhtunkhwa in 2008 (Halstead 1988, Fontenille, Failloux *et al.* 2007). The mosquito *A. aegypti* flourishes on contaminated water lay eggs and feed on human blood thus transmitting dengue virus into the person. The mosquito bites leads to dengue fever characterized by 5-7 days fever, headache, stomach ache, rash, myalgia, arthralgia or dengue hemorrhagic fever (DHF) with bleeding abnormalities, thrombocytopenia, increased vascular permeability, circulatory failure leading to death (Guzman and Kouri 2003, Hesse 2007, Haroon, Jan *et al.* 2018).

The recent outbreak in Peshawar, Pakistan claimed more than 60 lives and thousands of peoples were hospitalized. In the current study, we investigated the prevalence of DF and the hematological changes associated with the infection in district Mardan, KP Pakistan.

Materials and methods

The current study was conducted in Mardan Medical Complex (MMC). The ethical approval for the study was obtained from the ethical committee of Bacha Khan University, Charsadda, KP and from the medical superintendent of the MMC. The duration of the study was from August 2017 to November 2018.

Study area

In district Mardan, the blood collected from suspected individuals belongs to Katlang (188 cases), Mardan city (263 cases), Takhtbhai (225 cases), Rustam (174 cases) and Lund Khwar (150 cases).

Sample size and inclusion criteria

In the current study, 1000 samples were collected from the patients having a fever and other clinical symptoms from 7 days. A specially designed semi-structure questionnaire form was used to collect the data on the demographic factors such as age, gender, location, health conditions and exposure to possible risk factors.

Laboratory procedure

The blood collected was centrifuge at 3000 rpm for 5 minutes. The extracted serum was stored at -80°C until further use.

Primary infection and secondary infection

Primary and secondary infection of dengue was detected by using ICT strips which were IgM and IgG antibody coated and 5ul of the suspected individual's sera was added to the well with the addition of buffer. The strips were then placed at room temperature for 15 min. The positive and negative infection were distinguished by the development of red line in the IgG and IgM region just like development in the control region in case of positive infection. While in

case of negative infection the line will develop in the only control region.

NS1-Antigen detection

For the identification of Dengue NS-1 antigen in the patient serum, NS1 antigen ICT strips were used. The suspected individual sera (5 μl) was added to the well with the addition of buffer. The strip was then placed at room temperature for 15 min. The positive and negative infection were distinguished by the development of red line in the NS-1 region and in the control region respectively.

Complete blood count (CBC)

The CBC was determined for patients undergoing hypo-volumetric shock condition. The blood profile was determined by ALERE-380 blood cell counter

machine. The main components of blood such as HB, HCT, PLT, and TLC values were recorded from all suspected patients.

Statistical analysis

The results obtained were statistically analyzed with SPSS version 21 (IBM SPSS Statistics v21) into groups and subgroups on the basis of their age and gender. The analysis of the blood profile in the patients was also done.

Results

A total of 1000 dengue suspected individuals were examined, out of which 134 (13%) patients were infected with dengue virus. In the dengue positive patients, 88 (66%) were male and 46 (34%) were female as shown in Table 1.

Table 1. Gender-wise distribution of dengue infection.

Gender	Positive	Negative	Total
Male	88	544	632
Female	46	322	368
	134	866	1000

Prevalence in various age groups

The prevalence of the dengue infection was determined in various age groups and for which patients were classified into 4 groups (Fig. 1). The highest prevalence was observed in the age group of

36-50 years with total 48 (36%) cases followed by age group 61-70 years with 41 (30%) cases. The frequency of dengue infection was 24 (18%) and 21 (16%) in 16-35 and 1-15 years age groups respectively.

Table 2. Laboratory markers of dengue virus infection.

Gender	NS1	IgG	IgM
Male	20	19	49
Female	10	15	21
Total	30	34	70

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 49 male and 21 female patients. IgG antibody alone was the second most prevalent with 19 male and 15 female

patients cases. NS1 antigen was observed in 20 male and 10 female patients as shown in Table 2.

Blood profile of dengue-infected patients

Different dengue markers including the NS1 antigen, IgM and IgG antibodies were determined individually with specific ICT strips. In these patients, the blood profile was determined separately for each marker. The results are shown in Table 3 and Table 4.

Table 3. Blood profile of dengue-infected patients (Male patients).

Male Total: 88	NS1 antigen Positive: 20	IgM antibody (Primary infection) Positive: 49	IgG antibody (Secondary infection) Positive: 19
Hypo volumetric condition	Positive: 10 Normal: 10	Positive: 10 Normal: 39	Positive: 9 Normal: 10
TLC	Low: 5 Normal: 5	Low: 3 Normal: 7	Low: 5 Normal: 4
Platelets	Low: 8 Normal: 2	Low: 4 Normal: 6	Low: 6 Normal: 3
Hb	High: 3 Normal: 7	High: 1 Normal: 9	High: 3 Normal: 6
HCT	High: 3 Normal: 7	High: 2 Normal: 8	High: 3 Normal: 6

NS1 antigen: In NS1 antigen positive male cases 10 out of 20 patients undergoes hypo- volumetric shock condition. The total leukocyte count (TLC) test and platelet count was conducted for these cases and 5 patients were having low TLC values and 8 patients were having low levels. The hemoglobin and HCT levels were elevated than normal range in 3 cases each. Similarly, in total 46 positive dengue female patient CBC of 10 patients were also determined which undergoes hypo volumetric shock condition. Out of 10 positive patients, 4 patients were having low TLC and 5 patients were having low platelets count. While the hemoglobin level of 1 patient was high while the HCT value of 3 patients was high.

Table 4. Blood profile of dengue-infected patients (Female patients).

Female Total: 46	NS1 antigen Positive: 10	IgM antibody (Primary infection) Positive: 21	IgG antibody (Secondary infection) Positive: 15
Hypo volumetric condition	Positive: 10 Normal: 0	Positive: 10 Normal: 11	Positive: 8 Normal: 7
TLC	Low: 4 Normal: 6	Low: 8 Normal: 2	Low: 4 Normal: 4
Platelets	Low: 5 Normal: 5	Low: 5 Normal: 5	Low: 4 Normal: 4
Hb	High: 1 Normal: 9	High: 1 Normal: 9	High: 2 Normal: 6
HCT	High: 3 Normal: 7	High: 2 Normal: 8	High: 1 Normal: 7

IgM antibodies: In the dengue suspected cases the primary infection was determined with IgM antibodies strips. Out of the total of 88 positive dengue male patient, 49 patients were IgM positive.Then CBC of 10 male and 10 female patients were determined which undergoes hypo volumetric shock condition. Low TLC levels were observed in 8 female and 3 male patients. The platelets count were low in 4 male and 5 female patients. While the level of hemoglobin observed was high in very few cases. The HCT level was high in 3 male and 3 female patients.

IgG antibodies: In the dengue suspected cases the secondary infection was determined with IgG antibodies strips. The IgG antibodies were detected in 19 male and 15 female patients. CBC of 10 male and 10 female patients was conducted which undergoes

hypo volumetric shock condition. The TLC and PLT levels were low in 5 and 6 patients respectively. While the HB and HCT levels were high in 3, 3 patients.

Discussion

In the current epidemiological study the seroprevalence of the dengue infection in district Mardan, KP along with the hematological changes in the infected individuals. A total of 1000 dengue suspected individuals were examined, out of which 134 (13%) patients were infected with dengue virus which included 88 (66%) male and 46 (34%) female cases.

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 followed by the IgG antibody and NS1 antigen.

The results were in accordance with a similar study conducted in Peshawar, KP in which IgM antibody alone was more prevalent in the majority of the cases. IgG antibody alone was the second most prevalent followed by NS1 antigen alone (Haroon, Jan *et al.* 2018).
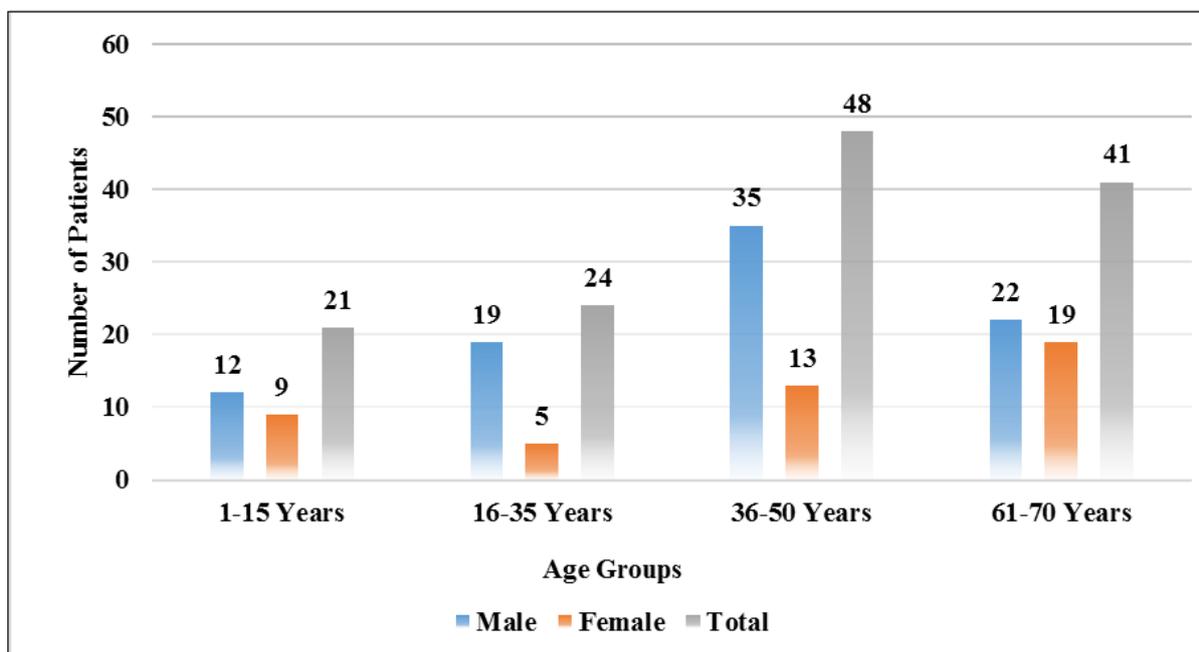
Fig. 1. Prevalence in various age groups.

Hematological changes were also observed in dengue infected patients. The dengue positive patients which undergo hypo-volumetric condition were further examined for the abnormalities in TLC, platelets, Hb and HCT levels. The changes were determined separately for NS1, IgM, and IgG positive cases individually. With dengue fever, both the white blood cell count and the platelet count decreases. In previous studies patients with dengue shows vascular permeability and thrombocytopenia leading to plasma leakage and bleeding tendency which leads to elevated Hb and HCT value (Srichaikul and

Nimmannitya 2000, Tsai, Chang *et al.* 2017). In the current study, the platelet count was observed low in 56% of the cases followed by low TLC level in 50% of cases. The HCT levels were abnormally high in 24% of the cases followed by high Hb level in 19% of the cases.

Conclusion

The current study provides the frequency of dengue infection and evidence for hematological changes in dengue virus-positive patients in district Mardan, Khyber Pakhtunkhwa (KP). The assessment and

comparison of laboratory markers, clinical symptoms, and hematological profiles play a significant role during the epidemiological studies of dengue infection. Extensive research is of utmost importance for the development of efficient, reliable and accurate diagnostic technique and to build prominent prophylactic strategies to minimize the consequences of the dengue disease.

Authors Contribution

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

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 Mardan Medical Complex (MMC), KP. Funding.

Competing interest

None declared.

References

Ali A, Ahmad H, Idrees M, Zahir F, Ali I. 2016. Circulating serotypes of dengue virus and their incursion into non-endemic areas of Pakistan; a serious threat. Virology journal **13(1),** 144. https://doi.org/10.1186/s12985-016-0603-6

Cardier JE, Mariño E, Romano E, Taylor P, Liprandi F, Bosch N, Rothman AL. 2005. Proinflammatory factors present in sera from patients with acute dengue infection induce activation and apoptosis of human microvascular endothelial cells: possible role of TNF-α in endothelial cell damage in dengue. Cytokine **30(6),** 359-365. https://doi.org/10.1016/j.cyto.2005.01.021

Chen RF, Yang KD, Wang L, Liu JW, Chiu CC, Cheng JT. 2007. Different clinical and laboratory manifestations between dengue haemorrhagic fever and dengue fever with bleeding tendency. Transactions of the Royal Society of Tropical Medicine and Hygiene **101(11),** 1106-1113. https://doi.org/10.1016/j.trstmh.2007.06.019

Diamond MS, Pierson TC. 2015. Molecular insight into dengue virus pathogenesis and its implications for disease control. Cell **162(3),** 488-492. https://doi.org/10.1016/j.cell.2015.07.005

Fontenille D, Failloux AB, Romi R. 2007. 10. Should we expect Chikungunya and Dengue in Southern Europe? Emerging pests and vector-borne diseases in Europe, 169.

Guzman MG, Kouri G. 2003. Dengue and dengue hemorrhagic fever in the Americas: lessons and challenges. Journal of Clinical Virology **27(1),** 1-13. https://doi.org/10.1016/S1386-6532(03)00010-6

Halstead SB. 1988. Pathogenesis of dengue: challenges to molecular biology. Science **239(4839),** 476-481. http://dx.doi.org/10.1126/science.239.4839.476

Haroon M, Jan H, Faisal S, Ali N, Kamran M, Ullah F. 2018. Dengue Outbreak in Peshawar: Clinical Features and Laboratory Markers of Dengue Virus Infection. Journal of infection and public health. https://doi.org/10.1016/j.jiph.2018.10.138

Hesse RR. 2007. Dengue virus evolution and virulence models. Clinical Infectious Diseases **44(11),**

1462-1466.
https://doi.org/10.1086/517587

Srichaikul T, Nimmannitya S. 2000. Haematology in dengue and dengue haemorrhagic fever. Best Practice & Research Clinical Haematology **13(2),** 261-276.
https://doi.org/10.1053/beha.2000.0073

Tsai JJ, Chang JS, Chang K, Chen PC, Liu LT, Ho TC, Tan SS, Chien YW, Lo YC, Perng GC. 2017. Transient monocytosis subjugates low platelet count in adult dengue patients. Biomedicine Hub **2(1),** 8-8.
https://doi.org/10.1159/000457785