

ORIGINAL ARTICLE

Epidemiological Parameters of Dengue Infections in Pakistan

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ABSTRACT

Objective: The aim of this research work was to find out epidemiological features associated to age, nature of prevalence, serology and severity of the disease.**Methodology:** This study was conducted in Benazir Bhutto Hospital; Rawalpindi and the duration of this study was from June 2021 to November 2021. We collected total 149 specimens of sera of the suspected patients appearing with the symptoms of dengue. We analyzed the gathered samples with the help of serological detection of IgM (Immunoglobulin-M), IgG (Immunoglobulin-G), RT-PCR (Reverse transcriptase-Polymerase chain reaction) and culture of virus in Cellline C36/36.**Results:** Findings of this research work showed that out of total one hundred and forty-nine specimens, 32.20% (n: 48) were identified with dengue virus with the use of RT-PCR & cell culture and 72.50% (n: 108) samples were found positive by serology. Of one hundred and eight cases, 26.80% (n: 48) patients were IgM positive, 41.0% (n: 61) were both IgM & IgG positive and 4.70% (n: 07) patients were IgG positive only. There was very high prevalence of dengue in age-group of patients having 20-29 years of age and the incidence of the infection was much high in male patients as compared to females. There was highest epidemic intensity in month of June as compared to other months.**Practical Implication:** This current research makes it possible to classify the serological results of dengue by mixed primary-secondary, primary and secondary dengue infections. There is also relation of dengue infection with the season as there is high activity of this infection after season of monsoon**Conclusion:** The prevalence of dengue is very endemic in our country Pakistan and the most vulnerable age group was 20-29 years for this infection. There is strong need of the effective prevention as well as control measures for the reduction of this infection.**Keywords:** Infection, serology, dengue, RT-PCR, IgM, epidemiology, IgG, prevention, prevalence, incidence, endemic.

INTRODUCTION

Dengue is a virus which is under Flaviviridae family. There are 4 serotypes of dengue virus from Den-1 to Den-4. All these serotypes have the capability to infect the human beings and main cause of development of DF (Dengue Fever), DHF (Dengue Hemorrhagic Fever) and DSS (Dengue Shock Syndrome) ⁽¹⁾. Getting infection of one type is not able to confer the immunity for other serotypes but it may cause other serious complications ⁽²⁾. According to one report stated by WHO in 2009, it was concluded that there are more than fifty million dengue infections every year in whole world. The mount of the dengue patients in our country is very high. There is a highest amount of the patients and mortality reported for 4 countries in West Pacific Region in years 2001 & 2008 ⁽³⁾. In our country Pakistan, A. aegypti dengue virus is more common in urban regions and A. Albopictus is very common in peri-urban areas. DF and DHF are highly associated with the A. Aegypti in urban regions. But there was no satisfying evidence to associate the relationship of A. Albopictus with the severe condition of the dengue infection ⁽⁴⁾. DHF and DF are the most important health problem in our country Pakistan as there are many outbreaks of these complications before ⁽⁵⁾.

Dengue is quite devastating public health issue frequently encountered in urban slums as well as semi-urban zones of globally endemic regions. Ranawaka et al., found that greater than 70% of dengue infections detected in Asian countries, case fatality rate is anticipated to be 20% without appropriate case management. In major cities of Punjab particularly in Lahore, dengue cases were drastically escalated in last week of September 2021. Periodic surveillance of at-risk places like tyre shops and graveyards was carried out by the concerned socials for fumigation and drainage of stagnant water in order to eliminate the mosquito breeding places. These measures in addition to awareness campaign are of paramount significance in order to prevent the repetition of dengue epidemic 2019 scenario. In comparison with our research, Indian statistics pertinent to dengue showed crowning of cases in September 2021 that was determined to be about 217. However, dengue cases in

September 2020 amidst peak of COVID pandemic were approximately 188 revealing fewer predilections for dengue last year ⁽⁶⁾. On the other hand, drastic reduction in dengue cases in Malaysia was observed from 63,988 during Jan-Aug 2021 to 16,565 during the same tenure in 2021. As there was no vaccine against dengue, so strict adherence to preventive strategies was the only option to mitigate the chances of dengue epidemic at this crucial moment when our frontline warriors are already confronted with COVID pandemic associated healthcare challenges. Pan American Heart Organization (PAHO) verified the occurrence of 416,289 dengue cases till 18th September 2021 and approximately 265 dengue related deaths in various states of America. The countries revealing the highest propensity of dengue cases during 2021 are Brazil, Peru, Nicaragua, Colombia and Mexico. Likewise, Pakistan, rise in dengue cases was spotted in other Asian countries including Bangladesh, Cambodia and Laos ⁽⁷⁾. The rapid spread of bacterial and viral infections across the world has become possible due to globalization. Already sufficient health resources in our healthcare facilities were spent in investigating and managing the COVID menace since March 2020; it will be impossible for our healthcare professionals to manage the exceeding dengue cases along with COVID havoc in public sector hospitals due to limited budget and staff shortage. Tan et al., found that most of the dengue cases were noticed across the globe but still this infection constitutes the submerged portion of disease iceberg due to underreporting or misdiagnosis of most of the cases. In view of emerging and re-emerging infections, World Health Organization is seriously committed to pledge with other countries for adequate preparedness in order to ght against Public Health Emergencies of International Concern (PHEIC) ⁽⁸⁾. Even one of the ten issues declared by WHO to track in 2021 was to curtail the spread of communicable diseases like polio, TB, malaria etc.

There are many observations of outbreaks of DHF and DF in our country Pakistan Before ⁽⁶⁾. Our country faced the worst outbreaks in 1983. Fang in his research study conducted in 1884 stated more than three thousand patients and thirty five deaths due to DHF and DF and most of the deaths occurred in June to

September. Epidemiological design of this infection is changing in our country Pakistan as a consequence of increase in number of the infected patients every year. The current rate of incidence of dengue infection has reached at 64.50 per 1, 00,000 persons^[(9)]. Extensive urbanization is in favor of the high prevalence of dengue infections because of the formation of suitable breeding regions for mosquitoes. Kobayashi in his research study stated that Den-3 was endemic in Thailand which further spread to other regions of world^[(10)].

METHODOLOGY

This study was conducted in Benazir Bhutto Hospital; Rawalpindi and the duration of this study was from June 2021 to November 2021. All the patients present with DF, DHF and DSS were the participants of this research work who were fulfilling the clinical definition^[(11)]. Ethical committee of the institute gave the permission to conduct this research work after knowing the purpose of this study. We collected one hundred and forty nine samples of blood from the patients of different age groups. We recorded the gender, age, time and severity of disease for all the patients. We drew 3 to 5 milliliter venous blood from every patient appearing with the symptoms of dengue infection. We allowed the blood to clot at normal temperature for complete one hour,

Table-1: Primer Sequence Generated by C-prM

No	Primer	Primer Sequence	Specificity	Serotype	Primer set	Product size (bp)
1	mD1	TCAATATGCTGAAACGCGAGAGAAACCG	DENV (all)			
2	D2	TTGCACCAACAGTCAATGTCTTCAGGTTTC	DENV (all)		mD1-D2	511
3	rTS1	CCCGTAACACTTTGATCGCT	DENV-1	DENV-1	mD1-rTS1	208
4	mTS2	CGCCACAAGGCGCATGAACAGTTT	DENV-2	DENV-2	mD1-mTS2	119
5	TS3	TAACATCATCATGAGACAGAGC	DENV-3	DENV-3	mD1-TS3	288
6	rTS4	TTCTCCCCTTCAGGATGTTTC	DENV-4	DENV-4	mD1-rTS4	260

The rate of incidence of dengue was very high in the age group of 20-29 years. This infection was much common in male gender as compared to females. Epidemic intensity of this infection was higher in the month of June and least infection was recorded in the month of August.

Table 2: One step RT-PCR Amplification

Steps	Temp.	Time	No. of Cycle
Preheated thermal cycle	50°C		
Reverse Transcription :	50°C	30 min	1
Initial PCR activation step :			
- Denaturation	95°C	15min	1
- Annealing	55°C	15second	1
- Extension	72°C	30 second	1
Denaturation :	95°C	15 second	34
Annealing :	55°C	15 second	34
Extension :	72°C	30 second	34
Final Extension	72°C	10min	1
Hold	4°C		

Serology determined the gender distribution of infections related to dengue. We observed that there was a high prevalence of this infection in male gender as compared to females. The collected information showed that infection in male was 63.0% (n: 68) and among females as 37.0% (n: 40).

Table 3: Semi-Nested PCR Amplification by Hot Start Taq Polymerase

Steps	Temp.	Time	No. of Cycle
Activation Taq polymerase	95°C	15min	1
Denaturation	95°C	15second	25
Annealing	55°C	15 second	25
Extension	72°C	30 second	25
Final Extension	72°C	30 second	1
Hold	4°C		

On the basis of serology, we found that primary dengue was available in twenty eight samples, secondary dengue was available in seven samples and sixty one samples were found as positive for

centrifuged the clotted blood at 3500.0 rpm for complete five minutes and collection of the serum was carried out and stored at a temperature of -21 centigrade for use further.

We used the SD BIOLINE dengue IgG and IgM rapid test kit for the analysis of antibodies of dengue. This kit separated the all four serotypes of dengue. We followed the Chew procedure for cell culture^[(12)]. We checked the used slides with the help of fluorescent microscope. QI Amp RNA kit was used for the extraction of total RNA following the protocols of manufacturers^[(13)]. Following the procedure of Chew, RT-PCR was in use for the identification of all four serotypes causing dengue infections. We used the gel electrophoresis for the PCR product's visualization.

RESULTS

The primer sequence generated by C-prM is shown in Table-1. Table-2 presents the results of this current research study. Out of total one hundred and forty nine examined samples of sera, 32.20% (n: 48) samples were positive for dengue virus by RT-PCR & cell culture and 72.50% (n: 108) samples were found positive by the serology. Among these one hundred and eight patients, 26.80% (n: 40) samples were IgM-positive, 41.0% (n: 61) samples were both IgM & IgG positive and 4.70% (n: 7) patients were only IgG-positive.

both primary as well as secondary dengue, this finding showed that sera of these patients provided the positive results for both IgG and IgM. In infection of primary dengue, twenty eight patients were male and twelve patients were from female gender. In infections of secondary dengue, one patient was male and six patients were females. In mixed reaction, twenty two were females and thirty nine patients were males. During the period of this research work, we observed that highest amount of the patients were documented in the month of June (n: 32), then September (n: 22) and October (n: 22). Least number of patients were available in the month of August (n: 6).

Table-4: Detection of Dengue Virus by Cell Culture, RT-PCR and Serology in Relation to Age Group At UKMMC.

Age Group (years)	Culture and RT-PCR positive	Serology Assay*			Total (%)
		IgM Positive	IgM and IgG positive	IgG positive	
0-9	1	2	1	0	4(2.6)
10-19	16	10	14	0	40(25.6)
20-29	15	20	16	3	53(34.6)
30-39	6	5	11	2	24(15.4)
40-49	6	0	8	0	14(9.0)
50-59	4	3	4	1	12(7.7)
60-69	0	0	5	0	5(3.2)
70-79	0	0	2	1	3(1.9)
Total	48	40	61	7	155+(100)

Legend: * Dengue IgM and IgG rapid test (SD BIOLINE, Korea)

DISCUSSION

This current research makes it possible to classify the serological results of dengue by mixed primary-secondary, primary and secondary dengue infections. There is no research work in our country which can classify the dengue infections. This research work showed that the prevalence of all types of dengue infections was 71.80%. Some other research work conducted by different professionals on the incidence of serological dengue infections found the prevalence as 45.0% in Singapore, 66.0% in Peru, 69.0% in Brazil and 78.0% in Delhi a famous city in India^[(14,15,16,17)]. The

prevalence rate found in this research work was next higher to the rate of prevalence found in India. The most vulnerable age group was consisting the patients having 20 to 29 years of age. The prevalence was more common among males as compared to female patients in our country Pakistan. Epidemic intensity was very high in the month of June and lowest epidemic intensity was found in the month of August. The high incidence of this infection in this particular group was because of more activities in outdoors which caused the young adults more exposure to mosquitos. Same findings were the results of the research work conducted by Sigh & Ang^[(18)] who also stated the high amount of patients in young adults and patients in their middle ages.

The incidence was much low in the upper age group because of high development of immunity due to previous exposure. This current research work showed that there were 63.0% (n: 68) males and 37.0% (n: 40) female patients as diagnosed in this research work. The reason was the more travel and much activities outside which make them exposed to the mosquitoes. Goh^[(19)] in his research work also stated that there was high prevalence of dengue infection in males as compared to females. When analyzing the epidemiological data in various months of this study, we observed that there were thirty two patients in the month of June and twenty two patients in the months of October and September. We found the least amount of patients in the month of August (n: 6). It was very hard to explain the high amount of patients in the months of June but one estimation is that there is more rain in the month of June which favors the growth of larva as providing breeding sites in shape of stagnant water. There is also relation of dengue infection with the season as there is high activity of this infection after season of monsoon^[(20)].

CONCLUSION

Dengue is very significant issue for public health in our country Pakistan. This research work showed that adult population was more vulnerable to this infection. The modification and improvement in the control programs for the prevention from dengue infection are very important. There is need for the development of reliable tetravalent vaccine for all the 4 serotypes of dengue virus which can easily mitigate this serious health issue.

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