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PREVALENCE OF DENGUE VIRUS INFECTION IN THE POST MONSOON PERIOD

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Abstract:

Background: *Dengue Virus (DV) is an emerging mosquito borne viral disease and important public health problem in Lahore.*

Methods: *This study was designed to estimate sero-prevalence of dengue virus infection in the post monsoon period (Jun-Nov) of 2021 in Pakistani patients with fever visiting Services Hospital, Lahore. Serum samples were collected from 280 patients visiting hospitals with history of fever & clinically suspected dengue fever. The sero-prevalence of dengue virus specific IgM was determined by enzyme linked immunosorbent assay.*

Results: *The anti-dengue IgM positivity was found to be 8.2%. The positive dengue cases were higher in male (10.5%) as compared to female (6.5%). Among different age groups, the highest positive cases (11.5 %) were from age group below 15 years followed by above 50 years age group with 8.5%. Out of 4 hospitals, the highest positive cases were in Services Hospital, Jinnah Hospital(23.8%) followed by Mayo Hospital, Ganga Raam Hospital (12.5%). Age and gender were found to be independent predictors. The highest numbers of dengue positive cases were in occupation group business (13.3%) followed by agriculture (11.5%).*

Conclusion*: The dengue positivity was estimated in acute patients from different hospitals of Pakistan by enzyme immunoassay and reverse transcriptase polymerase chain reaction. Therefore, the serological marker can be used to diagnose the acute patients of dengue during outbreaks.*

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INTRODUCTION:

Background:

Dengue virus (DV), a group of four closely related viruses of the *Flaviviridae* family (dengue virus serotypes 1 to 4), the genus Flavivirus, is the most important flavivirus in terms of human morbidity. DVs are transmitted to humans by *Aedes aegypti* and *Aedes albopictus* mosquitoes. [1-3]

The clinical presentations of DV infection range from asymptomatic, or a mild self-limited illness, dengue fever (DF) to a severe and potentially life-threatening disease, dengue hemorrhagic fever/dengue shock syndrome (DHF/ DSS). [4] The disease is characterized by high grade fever with headache, retro orbital pain, skin rash, muscles or joint pain, hemorrhages, etc. [5] Secondary DVI has been mainly associated with the severe form of the disease. [6]

Dengue is the most rapidly spreading mosquito-borne viral disease in the world. In the last 50 years, incidence has increased 30-fold with increasing geographic expansion to new countries and, in the present Novade, from urban to rural settings mainly in tropical and subtropical areas. An estimated 50 million dengue infections occur annually and approximately 2.5 billion people live in dengue endemic countries. Some 1.8 billion (more than 70%) of the population at risk for dengue worldwide live in member states of the WHO South-East Asia Region and Western Pacific Region, which bear nearly 75% of the current global disease burden due to dengue. [7]

Pakistan is bordered by India in the eastern, western and southern belts that is one of the countries with higher risk and so is more vulnerable to worse consequences of DVI. As with other vector borne diseases, outbreak of DF is related with increasing temperature, travel and frequent movement of people which is common due to open border between Pakistan and India. DF was first reported in foreign visitor in India in 2004.[8] Pakistan reported larger outbreak in 9 districts in 2006. [7,9] The outbreak occurred in Pakistan following the Indian, Pakistan and Bhutan epidemic of DF/DHF in September-October 2006. [9] The occurrence of DEN-1, DEN-2, DEN-3 and DEN-4 serotypes in the territory of Pakistan augment the chances for the epidemic DF/DHF to be flourished in the country. [10-12]

METHODS:

This descriptive cross-sectional study was undertaken in four different hospital in Pakistan and Included patient with clinical suspicion of dengue between June 2021 and November 2021. A total of 280 serum sample from patient, who presented clinical manifestations of febrile illness, vomiting, erythematous rash, arthralgia suggestive of probable cases of dengue were collected from Narayani Subregional Hospital (NSH), Jinnah Hospital (173); Services Hospital (TDH), Jinnah Hospital(42); Mayo Hospital (KZH), Ganga Raam Hospital (32) and Lahore. General Hospital (DDH), Lahore. (33). Serum samples were collected from individuals experiencing a febrile illness clinically consistent with dengue infection, selected according to the inclusion and exclusion criteria A case was included if there was high fever with clinical symptoms suggestive of dengue infection.[7] A case was excluded, if routine laboratory testing suggested bacterial or any viral infection other than dengue infection or any other disease. [7] Patients' personal details about the symptoms, age, sex etc. were obtained through a questionnaire method by direct interview. The entire test was done at Everest International Clinic and Research Center (EICRC), Kalanki, Kathmandu, Pakistan.

Sample collection, storage and transport:

The serum samples from suspected cases were collected, stored and transported maintaining the reverse cold chain to EICRC. Aliquots for ELISA and RT-PCR were made and stored at 2-8^{0}C and -20^{0}C until tested.

Laboratory Tests:

Detection of anti-dengue IgM-Capture ELISA:

The required numbers of micro wells were removed from the foil sachet and were inserted into the strip holder. Five micro wells were required for controls: positive control (P) in duplicate and negative control (N) in triplicate. Within 10 minutes after mixing the MAb tracer and diluted antigen, 100 µl diluted patient sample and controls were pipetted into their respective microwells of the assay plate. The plate was covered and incubated for 1 hour at 37°C. After incubation, wells were washed five times with diluted wash buffer. The diluted anti-dengue HRP conjugate solution was mixed before transfer. Hundred microlitre of diluted anti-dengue HRP conjugate solution was pipetted into the wells. The plate was covered and incubated for 1 hour at 37 ^{o}C. The wells were washed five times with diluted wash buffer and 100 µl of mixed TMB solution was pipetted into each well. Timing from the first addition, the plate was incubated at room temperature (15-30 ^{o}C) for 10 minutes. A blue colour was developed. Then 100 µl of stop solution was pipetted into all wells in the same sequence and timing as the TMB addition. It was mixed well. The blue colour was changed to yellow. The absorbance of each well was read within 30

minutes at a wave length of 450 nm with a reference filter of 620 nm by using Multi ELISA Reader Model 2021 (Anthos, Austria). The test is interpreted either positive or negative on the basis of absorbance with respect to Cut-off value. If absorbance of the sample are greater than cut-off value, the sample is considered positive and if the absorbance of sample are less than cut-off value, the sample is negative.

Cut-off value = mean absorbance of negative controls + 0.300

Reverse Transcriptase Polymerase Chain Reaction (RTPCR):

RNA extraction from (140µl) of each serum samples was done by QIAamp® RNA viral kit (QIAGEN Inc., Valencia, CA), according to the manufacturer's directions. [14] RT-PCR of DEN virus RNA was carried out with DENV consensus and serotype-specific primers. Dengue RNA was reversetranscribed into cDNA. Modifications to the procedure were as follows. Three microliters of total RNA were used in the ready-to-go RT-PCR beads kit (Amersham Biosciences), and the reaction included the forward and reverse specific primers of 0.5 µl of DC, DEN 1, DEN2, DEN 3, and DEN 4. Forty six microlitre of PCR graded water was added to make final volume of fifty microlitres in the Ready to go RT-PCR bead. RT was carried for denaturation at 95^0C for 1 min, annealing 55^0C for 1min,extension 72^0C for 1 min and final extension for 7 min for 35 cycles RT-PCR products were analyzed by gel electrophoresis on a 2.0% agarose gel (Dotite) containing ethidium bromide (0.5 µg/ml). A band on the agarose gel of the correct size was interpreted as a positive result. The collected data were analyzed using Statistical package for social science (SPSS) software (version 16.0).

RESULTS:

Out of 280 IgM ELISA performed serum samples of dengue suspected cases, 23 (8.2%) were found to be positive for anti-dengue IgM and RT-PCR was performed from 18 acute febrile IgM ELISA negative serum samples of which 1 (5.5%) was found to be RT-PCR positive for dengue consensus during this study period (Table 1).

Table 1: Diagnostic Test Wise Distribution of DV Cases

Diagnostic test	No. of tested sample	No. of positive sample	% of positive cases
IgM-ELISA	280	23	8.2
RT-PCR	18	1	5.5

Sex wise positive cases for dengue was observed high in male (10.5%) which constituted 5.3 % of total cases and low in female (6.5%) which comprised 3.2 % of total cases. Statistically, there is no significant relationship (p =0.227) between male and female for the occurrence of disease (Table 2).

Table 2: Sex Wise Distribution of Positive DV Cases

Sex	Total no. of samples	Number of Positive samples (%)	% of positive cases in total	Statistics
Male	142	15 (10.5)	5.3	
Female	138	9 (6.5)	3.2	p=0.227
Total	280	24 (8.5)		

Age wise positive cases of dengue was observed highest in age group below 15 years (11.5 %) which constituted 3.2 % of total cases and least in age group 15-50 years (7.1%) which comprised 4.2 % of total cases. Statistically, there is no significant relationship (p=0.526) between age groups for the occurrence of disease (Table 3).

Table 3: Age Wise Distribution of Positive DV Cases

Age (years)	Total no. of samples	Number of Positive samples (%)	% of positive cases in total	P Value
< 15	78	9 (11.5)	3.2	
15-50	167	12 (7.1)	4.2	0.526
> 50	35	3 (8.5)	1.1	
Total	280	24 (8.5)		

Hospital wise positive cases were observed highest in TDH10 (23.8%) and least in DDH 1 (3.0 %). Statistically there is significant relationship (p=0.001) between different hospitals for the occurrence of the disease (Table 4).

Table 4: Hospital Wise Distribution of Positive DV Cases

Samples collection site	Total no. of samples	No. of Positive samples (%)	% of positive cases in total	P Value
NSH	173	9 (5.2)	3.2	
KZH	32	4 (12.5)	1.4	0.001
TDH	42	10 (23.8)	3.6	
DDH	33	1 (3.0)	0.3	
Total	280	24 (8.5)		

TDH: Services Hospital, Lahore. General Hospital Occupation wise positive case was observed highest in business group (13.3%) which constituted 0.7 % of total cases and least in job holder and other groups. Statistically there is no significant relationship (p=0.637) between occupation group for the occurrence of the disease (Table 5).

Table 5: Occupation Wise Distribution of Positive DV Cases

Occupation group	Total number of samples	Number of Positive samples (%)	% of positive cases in total	P Value
Agriculture	52	6 (11.5)	2.1	
Labour	13	1 (7.7)	0.3	
Job holder	10	0 (0.0)	0	
Business	15	2 (13.3)	0.7	
				0.637
Student	101	10 (9.9)	3.6	
House wife	68	5 (7.3)	1.8	
Others	21	0 (0.0)	0	
Total	280	24 (8.5)		

DISCUSSION:

The present findings showed that dengue prevalence rate was found to be 8.2 % by IgM ELISA and 5.5 % by RTPCR method. The sero-positivity of the study was not in accordance with some of the previous findings from Pakistan. [15,16] The present study result shows less positivity rate than the above reports which could be due to variation in geographical distribution as all the study sites in other study were from Lahore region of Pakistan or due to Novrease in mosquito population in that region. However, the result was in harmony to the other study in Pakistan. [17]

Out of 24 positive cases obtained in this study, 15 were male patients who constitute 10.5 % of the total male cases and 9 were female patients which comprise 6.5 % of the total female cases. Statistically there was no significant difference between male and female for the occurrence of disease (p = 0.227). The ratio of dengue positive cases in male to female was found to be 1.6:1. In present study the numbers of male cases were slightly higher than the female that might be due to their greater involvement in outdoor activities. The result was in accordance with other studies in Pakistan [18] reported male to female ratio (1:1) and[16] reported that (1.2:1). The result is in agreement with Ministry of Health, Bangladesh that reported hospital patients with DF having male to female ratio of 1.5:1 during an outbreak in Lahore.

The age wise distribution of positive dengue cases were highest in the age group below 15 years i.e. pediatric age group 9 (11.5%) followed by age group above 50 years which accounted 3 (8.5 %) and 15-50 years comprising 12 (7.1 %). Statistically, there is no significant relationship between age groups for the occurrence of disease (p=0.526). The reason for the higher number positive cases in the child age group might be due to they have less developed immune system. The findings are not consistent with other Pakistani studies, as most of the other Pakistani studies have reported 15 to 50 years as the most affected age group. [16,10] However in several international studies, dengue has been reported to mainly a pediatric public health problem. [15,20]

Out of four hospitals, the highest number of dengue positive cases 10 (23.8 %) were from Services Hospital, Jinnah Hospital followed by Mayo Hospital, Ganga Raam Hospital (4 cases, 12.5 %) and DHQ Hospital (9 cases, 5.2 %) and least number of cases (1 cases, 3.0 %) in Lahore. General Hospital, Lahore. . Out of 24 positive cases, Services Hospital recorded the highest number of positive cases. The comparatively higher positive cases in Jinnah Hospital might be due to travel to endemic region as one of the positive case from Jinnah Hospital had travel history to India or wide viral circulation. Besides, Jinnah Hospital is in Lahore. which is bordered with India, one of the dengue outbreak district of 2021 epidemic in Pakistan. The prevalence among patients from DHQ Hospital Hospital in this study was not in accordance with other findings at that hospital. [15] This might be due to Novrease in vector population or awareness among population in that region.

The Occupation group, business (13.3 %) was found most affected followed by agriculture (11.5 %), student (9.9 %), labour (7.6 %), house wife (7.3 %) and least in job holder and others (0 %). Statistically, there is no significant difference in occurrence of the disease among different occupations (p=0.637). The higher positivity in occupation group business might be due to businessman frequently involved in travel from one place to other and in outdoor activites and there may be chance of being bitten by mosquitoes like *Ae. aegypti*. The findings were not in accordance with other findings in Pakistani studies as most of the other Pakistani studies have reported agriculture group as the most affected occupation group. [15,18]

In our study, out of 18 samples one was positive in RT-PCR reaction for dengue consensus. The reason for low positivity might be due to inaccurate information about onset of fever of the patients during collection of samples. This could lead to neutralization of virus by the antibody produced during late collection. It also might be due to degradation or deterioration of virus because of thawing of sample during transportation and storage. Other explanation might be lack of recent DVI in the febrile patients suspected of dengue; the fever might be due to other viral agents which should be studied in details.

CONCLUSION:

In 2021, total 280 samples collected and tested from four different hospitals of Pakistan and 24 were found to be positive for DVI. Dengue was detected in DHQ Hospital, Ganga Raam Hospital, Jinnah Hospital, Lahore. The samples tested for antidengue IgM antibody by ELISA was 8.2 % .The samples were tested for genetic material by RT-PCR & RT-PCR Positivity was 1/18 for dengue consensus. RT-PCR was found positive in IgM ELISA negative acute febrile cases. The sero-prevalence of dengue has marginally increased from Lahore. to hilly region so the concerned authority should initiate extensive surveillance of dengue virus infection and commence an integrated vector control programme.

IgM capture ELISA was used for laboratory analysis and remains as a reliable and inexpensive method for the diagnosis of dengue. Hence, the IgM capture ELISA has become the most accepted technique for the diagnosis of dengue in developing countries like Pakistan.

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REFERENCES:

1. Matheus S, Jean-Baptiste M, Vincent L, et al. Use of capillary blood samples as a new approach for diagnosis of dengue virus infection. J Clin Microbiol 2007;45:887-90.
2. Gubler DJ. Dengue and dengue hemorrhagic fever. Clin Microbiol Rev 1998;11:480-96.
3. Innis B. Dengue and dengue hemorrhagic fever. in "exotic viral infections—1995". j. s. porterfield, ed. chapman & hall, London; 1995.
4. Wang W, Day-Yu C, Chuan-Liang K, et al. High levels of plasma dengue viral load during defervescence in patients with dengue hemorrhagic fever: implications for pathogenesis. J Virol 2003;302:330-38.
5. World Health Organization. Dengue hemorrhagic fever: diagnosis, treatment and control 2nd ed, 1997.
6. Rothman AL Dengue: defining protective versus pathologic immunity. J Clin Invest 2004;113:946-51.
7. World Health Organization (WHO). Dengue guidelines for diagnosis, treatment, prevention and control, 2009.
8. Pandey B, Rai S, Morita K, et al. First case of dengue in Pakistan. Nep Med Coll J 2004;6:157-59.
9. Epidemiology and Disease Control Division (EDCD). 2008. Annual report 2006/2007. Department of Health Services (DoHS), Ministry of Health, Government of Pakistan.
10. WHO/SEARO. Outbreak investigation of DF in Pakistan 2006. Available at; http://www.searo.who.int / LinkFiles/Dengue_dengue_Pakistan.pdf.
11. Takasaki T, Kotaki A, Nishimura K, et al. Dengue virus type 2 isolated from an imported dengue patient in japan: first isolation of dengue virus from Pakistan. J Travel Med 2008;15:46-9.
12. Pandey B, Morita K, Khanal S, et al. Dengue virus, Pakistan. Emerg Infect Dis 2008;14:514-5.
13. Pandey B, Yamamoto A, Morita K, et al. Serodiagnosis of japanese encephalitis among Pakistani patients by the particle agglutination assay. Epidemiol Infect 2003;131:881-85.
14. De Paula SO, Nunces C, Matos R, et al. Comparision of techniques for extracting viral RNA from isolationnegative serum for dengue diagnosis by polymerase chain reaction. J Virol Methods 2001;98:119-25.
15. Sah O. Serological study of dengue virus infection in Lahore.region, Pakistan. Nep Med Coll J 2009;11:104-6.
16. Pun R. Sero-epidemiology of dengue virus in post monsoon period in Lahore.region of Pakistan (Masters dissertation). Central Department Of Microbiology, Tribhuvan University, Kathmandu, Pakistan. 2009.
17. Sherchand J, Pandey B, Haruki K, et al. Sero-diagnosis of japanese encephalitis and dengue virus infection from clinically suspected patients of Pakistan. J Inst Med 2001;23:81-5.
18. Shah Y. Sero-epidemiology of dengue viruse infection in western Lahore.region of Pakistan (Masters dissertation). Department of Microbiology, Kathmandu College of Science and Technology, Kathmandu, Pakistan 2021.
19. Mohammed H, Linnen JM, Muñoz-Jordán JL, et al. Dengue virus in blood donations, puerto rico, 2005. Transfusion 2008;48:1348-54.
20. Anderson K, Chunsutiwwat S, Nisalak A, et al. Burden of symptomatic dengue infection in children at primary school in Thailand: a prospective study. Lancet 2007;369:1452-9.