Original Article

RETROSPECTIVE ANALYSIS OF DENGUE VIRUS INFECTION: A FOUR-YEAR STUDY

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ABSTRACT

Objective: To conduct retrospective surveillance study of dengue virus infection in patients presenting with febrile illness during 2015-2018.

Material and Methods: Dengue virus NS1 antigen and IgM antibodies were tested by ELISA method in serum samples of patients with febrile illness. Descriptive and analytical measures were used to assess the association of NS1 antigen and IgM antibodies with different risk factors like gender, age and month of the year.

Results: Over the period of four years (2015-2018), 2203 samples were received for dengue NS1 antigen. Out of these, 506 (23%) were positive and 1697 (77%) were negative. Among the positive samples, 162 (32%) were females and 344 (68%) were males, with significant association between NS1 antigen and gender ($p<0.05$). Similarly, 2089 samples were received for dengue IgM antibodies. Out of these, 389 (19%) were positive and 1700 (81%) were negative. Among positive samples, 128 (33%) were females and 261 (67%) were males ($p<0.05$). Moreover, significant association was observed for both NS1 and IgM cases with age. In addition, maximum number of positive patients for both NS1 and IgM were between 21-40 years of age. Most of the positive cases were detected during the months of July to October ($p<0.05$).

Conclusion: Dengue virus infection is a common cause of febrile illness in endemic countries like Pakistan. Male preponderance as well as high number of cases during rainy season were seen. Dengue infection was more prevalent among young people (21-40 years).

Key Words: Dengue, NS1, IgM.

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INTRODUCTION

Dengue is the most common vector-borne viral disease which was first reported in 1779 by David Bylon during an epidemic in Indonesia and was initially explained by Benjamin Rush as "break bone fever" in 1780 [1]. Dengue is a member of the family *Flaviviridae* and it has four serotypes. *Aedes aegypti,* the main vector of dengue, is a day time biting mosquito, which breeds in fresh water [2].

Typical symptoms of disease are abrupt onset of fever, followed by severe headache, retro-orbital pain, fatigue, severe myalgia and arthralgia. A maculopapular rash, often confluent with the sparing of small islands of normal skin has been reported [3]. This virus infects 50-100 million people per year while dengue hemorrhagic fever cases ranging from 20,000 to 500,000 annually. Its fatality rate is as high as 10% and can be reduced to as low as 1% if properly diagnosed and treated at an early stage [3] [4].

At present dengue is endemic in more than a hundred countries. Many outbreaks have been reported from all over the Asia including India, Sri Lanka, and Pakistan [5]. In Pakistan, dengue outbreaks have been reported in 1994, 1995 and 1997 in the past. After that, few sporadic cases were reported till the outbreak of 2006, which was followed by another outbreak from September to November in 2007 which was the cause of significant morbidity and mortality [2]. First major dengue outbreak occurred in 2004 in Pakistan, in which DENV-2 serotype was mostly reported [6]. According to the latest studies, the most predominant serotypes reported in Pakistan are DENV-2 and DENV-3 [7].

Historically, a number of strategies have been applied for diagnostic purpose. For example, virus detection by cell culture and immunofluorescence. Similarly, detection of virus antigen by enzyme-linked immunosorbent assay (ELISA), detection of anti-dengue virus antibody by hemagglutination inhibition (HI), complement fixation test (CF), neutralization tests and ELISA. Nucleic acid detection can be done by real-time reverse transcription-polymerase chain reaction (RT-PCR) [8-12]. Commercially available kits detect the presence of NS1 antigen or anti-dengue antibodies in the blood of suspected dengue patients. NS1 is basically a

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conserved glycoprotein present in serum samples of dengue affected patients, in high concentrations during the early clinical phase of disease, and is present from Day 1 up to Day 9 after onset of fever, in sample of primary or secondary dengue-infected patients. In primary infection, IgM can be detected on Day 3 to 5 of illness and may persist up to 6 months, whereas IgG appear by the fourteenth day and usually persists for life time [13, 14]. Commonly used diagnostic techniques in Pakistan are ELISA, PCR and rapid diagnostic tests [15].

The present study was conducted to retrospectively analyze the data of dengue IgM antibodies and NS1 antigen to find out the extent of febrile illness cases caused by dengue virus.

MATERIAL AND METHODS

Retrospective analysis of dengue NS1 and IgM tests was performed at Armed Forces Institute of Pathology (AFIP). The study duration was from January 2015 to December 2018. In this retrospective study, all the available data of tests performed during the study period, for Dengue NS1 and IgM was included. The serum samples were tested at the Virology department AFIP for the presence or absence of dengue specific IgM antibodies and NS1 Antigen. To test dengue-specific NS1 antigen, ELISA method was employed using Euroimmune Dengue NS1 kit (Germany) and Calbiotech Dengue IgM antibodies kit (Netherland). The instructions of the manufacturers were carefully followed while performing the tests.

The results available for a total of 2203 suspected cases of dengue tested for NS1 antigen and a total of 2089 suspected cases tested for IgM were compiled and analyzed. Qualitative variables were expressed by frequencies and percentages. Analytical tests, Fisher exact and Chi-square were used to assess the association of NS1 and IgM cases with risk factors. Results were considered significant at $p<0.05$.

RESULTS

Total number of samples for NS1 were 2203; out of these 506 (23%) were positive and 1697 (77%) were negative, as shown in Table 1.1. Among the positive samples, 344 (68%) were of males and 162 (32%) were of females ($p<0.05$), as shown in Figure 1.1 (a). In our study, no specific age group can be defined because samples from all ages were entertained. So age groups were distributed between the NS1 positive patients as shown in the Table 1.2. Maximum positive NS1 cases were between 21-40 years ($p<0.05$).

Similarly total numbers of samples received for IgM were 2089; out of these 389 (19%) were positive and 1700 (81%) were negative as shown in Table 1.1. Among positive samples, 261 (67%) were of males and 128 (33%) were of females, as shown in Figure 1.1 (b). Age group distribution between the IgM positive patients is shown in the Table 1.2. Maximum positive IgM cases were also in the age group 21-40 years ($p<0.05$). Year wise distribution of positive NS1 and IgM cases are shown in Figure 1.3, which shows that maximum cases were reported in 2015 ($p<0.05$). Overall, Significant association were observed between positive NS1 and IgM cases with gender, age and years ($p<0.05$).

Most of the positive cases of both NS1 and dengue IgM were reported from July to October, during each year of the study period, clearly showing seasonality of this viral infection in our area.

Table-1: Retrospective data of dengue NS1 antigen and IgM antibodies.

Cases	NS1	IgM
Positive	506 (23%)	389 (19%)
Negative	1697 (77%)	1700 (81%)
Total	2203	2089

Table-2: Age group distribution among NS1 antigen and IgM antibodies positive patients.

Age Groups	NS1		IgM	
	Males	Females	Males	Females
1-20	72 (14%)	18 (4%)	45 (11%)	22 (6%)
21-40	156 (31%)	69 (14%)	128 (33%)	59 (15%)
41-60	88 (17%)	54 (11%)	65 (17%)	36 (9%)
>60	28(5%)	21 (4%)	23 (6%)	11 (3%)
P-Value	0.032*	0.054	0.044*	0.621

Note: *p-value obtained using chi-square test, *$p<0.05$ showing significant association of NS1 and IgM +ve cases with age.

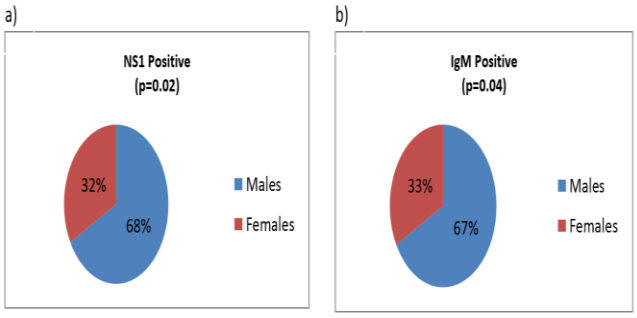

Figure-1 (a and b): Gender distribution in dengue NS1 and IgM positive patients, p-value obtained using Fisher Exact chi-square test, $p<0.05$ showing significant association of NS1 and IgM positive cases with gender.

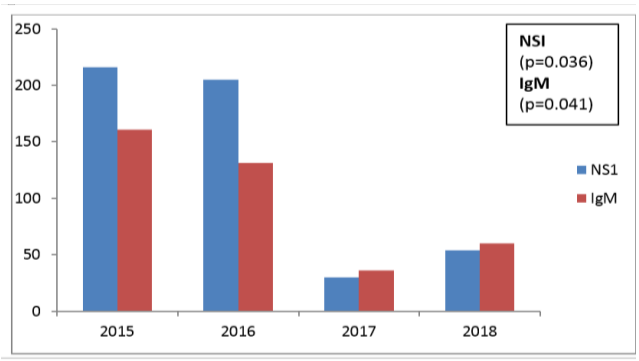

Figure-2: Year wise distribution of positive NS1 and positive IgM cases, p-value obtained using chi-square test, p<0.05 showing significant association of NS1 and IgM positive cases with years.

DISCUSSION

Dengue NS1 and IgM detection helps in early diagnosis of dengue virus infection [16]. In our study, mostly male population was affected from dengue fever. Male preponderance was seen in all age groups and this has also been reported in previous studies [17]. Proper body covering and clothes in our society as well as maximum indoor stay in females is a pertinent reason for the lower number of female cases [18].

Khan *et al* reported in 2007 about the dengue outbreak which occurred in 2006. In this study, they reported the demographic and clinical data of 172 IgM-positive patients [19] which is relevant to our study, where we have reported the data of 506 NS1 and 389 IgM positive patients. In an outbreak of dengue virus in Delhi (Gupta *et al*, 2003), a total of 811 cases were found to be IgM positive and maximum number of positive cases were in the age group 21-30 years. In this Indian study, most of the positive cases occurred in months of September to November [20], which is almost comparable to our study in which maximum cases were reported from July to October, a period of monsoon rains in our region. In another study, 841 positive cases were reported from Karachi; maximum cases were reported between 20 to 49 years of age. A gradual increase in dengue fever and dengue Haemorrhagic fever cases was seen in this study from August onwards, with a peak in October/November [21]. We have reported that the maximum number of positive patients for both NS1 and IgM, between 21-40 years of age, with similar pattern of seasonality.

Dengue fever shows seasonal variation in the South Asian region where vector population is maximum during monsoon period, with high rainfall and humid environment. Favorable climate provides excellent conditions for vector breeding [22] [23]. Mostly, high number of dengue cases are reported during the rainy season in our region [24]. A study carried out in Karachi (Khan *et al*) showed that the peak incidence of dengue fever was reported from August to October in Pakistan [19]. Gupta and colleagues also found the highest proportion of IgM positive dengue patients during the rainy season [20]. A similar trend was observed in our study about the seasonality of this viral infection.

CONCLUSION

It can be concluded from our study that Dengue virus infection is a common cause of febrile illness in endemic countries like Pakistan. Maximum cases of this mosquito borne diseases were reported during the rainy season (July-October). Male preponderance was seen as they are more involved in outdoor activities. Dengue virus infection was more prevalent among young people (21-40 years).

AUTHORS CONTRIBUTION

Humaira Aziz Sawal: Principal author, paper writing and results compilation.
Misbah Noor: Data collection and literature review.
Saifullah Khan Niazi: Paper writing, Literature review and final proof reading.
Eijaz Ghani: Overall supervision.
Sumaira Mubarik: Statistical analysis.
Noman Shakoor: Results compilation.

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