

EPIDEMIOLOGY OF DENGUE FEVER AND UTILITY OF DENGUE NS1 ANTIGEN RAPID DIAGNOSTIC POINT OF CARE TEST AT COMBINED MILITARY HOSPITAL MALIR

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ABSTRACT

Objective: To investigate the incidence and epidemiology of Dengue fever, and to evaluate the utility of NS1 antigen test for the rapid diagnosis of disease at Combined Military Hospital Malir, Karachi.

Study Design: Cross sectional prospective study.

Place and Duration of Study: Study was carried out at Combined Military Hospital Malir Cantt Karachi, from Jan to Dec 2018.

Methodology: Patients with acute febrile illness that reported to Combined Military Hospital Malir Cantt Karachi, Pakistan were enrolled in our study. The diagnosis was made on the basis of Dengue Serology by one or more of the following tests NS1 antigen, IgM or both by using ICT methods and a compatible clinical picture. The demographic characteristics, clinical features were noted.

Results: A total of 993 sera were collected from patients with suspected dengue infection out of which 89 were laboratory-confirmed dengue cases suggesting the incidence of dengue fever of 8.96%. Positive NS1 antigen alone was observed in majority of the cases with the frequency of 78 males and 07 females. NS1 antigen along with IgM antibody was observed in 1 male and 1 female only. Peak incidence was seen in the age group of 21-40 years i.e. 71%.

Conclusion: Diagnosis of Dengue Virus infection can be established early by keeping high suspicion for dengue in patients who presents with an acute febrile illness by using rapid diagnostic test that is dengue specific NS1 antigen along with IgM and IgG antibodies.

Keywords: Dengue Hemorrhagic Fever (DHF), Dengue Virus (DENV), Immuno Chromatographic Testing (ICT), NS1 antigen.

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INTRODUCTION

Dengue virus (DENV) infection has remained a great medical and public health concern date back since more than 200 years and many epidemics have been reported from many parts of the world particularly from Southeast Asia¹. As per the recent epidemiology dengue virus is responsible for around 50 to 100 million infections per year and over 2.5 billion individual at risk for infection². The vector responsible for transmission of virus are *Aedes aegypti* and *Aedes albopictus* but former are more widely distributed in South Asia primarily in India, Pakistan and Srilanka. Hyper endemic trans-

mission of all four serotypes is well established and outbreak have become more frequent³. Since many years DENV has been endemic in Pakistan for the reason of tepid climatic conditions of this region^{4,5} and various dengue outbreaks caused by different serotypes have been reported from this part of the world suggesting hyper endemic circulation^{6,7}. The activities of vector mosquito differs significantly because of the seasonal consequences in different geographical areas and dengue virus transmission is enhanced by rise in vector density, shorter mosquito incubation and amplified number of susceptible host⁸.

In the settings of epidemic transmission involving a single virus strain adults and children are most frequently affected and travelers are also at high risk of acquisition of infection whereas the frequency of Dengue Hemorrhagic Fever

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(DHF) is usually low, in past dengue virus almost exclusively followed this pattern but due to large population, increase travelling and more means of communication results in hyper endemic circulation which is a major factor contributing towards (DHF)⁹.

A provisional diagnosis of dengue fever is usually made on the basis of clinical signs and symptoms but require laboratory confirmation as the clinical features are indistinguishable from other infectious illnesses. There are various lab tools for the detection of virus e.g. nucleic acid detection (NAT), antigen/antibody assays and Viral culture¹⁰. Among these methods the most reliable test to diagnose an early infection are antigen detection, NAT testing and viral isolation by culture but the latter two options require specialized reference lab and high cost therefore the antigen detection remains the most convenient and cost effective lab diagnostic tool and by using Immuno Chromatographic Testing (ICT) technique results are available readily^{11,12}. NS1 antigen is detectable in blood from the very 1st day after the onset of fever and remains positive up to day 9, and is also detectable in the presence of IgM antibodies when viral RNA is negative by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)¹³.

The rationale of our study was to estimate the incidence of DENV infection in our setup by using rapid diagnostic technique which can lead to an early diagnosis and thereby reducing the morbidity and mortality associated with the disease.

METHODOLOGY

This cross sectional study was conducted in the department of Pathology in collaboration with the Medicine and Pediatrics departments, Combined Military Hospital (CMH) Malir Cantt Karachi, from January 2018 to December 2018. The study was approved by the Department of Research and Ethics committees CMH Malir (READ-IERB 19/01). Serum samples were collected during the study period. Our patient's population included serving /retired military

soldiers, officers and their families and persons from all age groups presenting to various departments of CMH Malir with acute febrile illness ($\geq 37.5^{\circ}\text{C}$). All patients were enrolled after getting the written consent during their first visit. Details of the patients with suspected dengue fever were noted using a structured proforma which included different sections for age, sex, ward, clinical details and date of blood collection was noted.

A total of 993 serum samples were collected, from patients with suspected dengue infection. Sample size was calculated using WHO calculator. Sampling technique was non probability consecutive sampling:

Using WHO sample size calculator with the following:

Confidence level: 99%

Anticipated population proportion: 0.656

Absolute precision required: 0.06

Sample size = $n=993$ patients

Descriptive statistics were calculated for both qualitative & quantitative variables, frequency and percentages were calculated and presented in the form of figures.

Dengue NS1, IgM and IgG serology was performed by using Immuno-chromatographic (ICT) kit (Merux USA). The manufacturer's instructions were followed in the procedure and results were interpreted as reactive and nonreactive.

Detection of Dengue NS1 antigen, IgM and IgG antibodies by ICT method:

There were separate strips used for Dengue NS1 Ag whereas a combined strip for IgM and IgG was used. These strips work on the principle of lateral flow immunochromatography for the detection of antigen and/or antibody. The recommendations from the manufacturer were followed which says that NS1 Strip to be read at 15 minutes and again at 30 minutes for doubtful results or negative results in patients with suggestive clinical features of dengue. In this study, the test was read at both 15 and 30 minutes for all

serum samples, and the results were analyzed separately. Technicians read each NS1 strip, IgM and IgG strip independently. If interpretations were conflicting, the final result was taken as equivocal.

RESULTS

There were 993 persons enrolled in the study out of these cases of dengue like febrile illness, 89 had laboratory confirmed DENV infection (positive by one or more of the diagnostic tests for DENV) suggesting the incidence of dengue fever of 89 (8.96%). Males (92%) were affected more than the females with a male to female ratio of 12:1. The patients suspected for DENV infection were tested for the presence of Dengue NS1 antigen and IgG plus IgM antibodies. NS1 antigen solely was most prevalent in the majority of the subjects with the frequency of 77 males and 06 females. The frequency of the presence of an IgM in 2 males and IgG was also seen in only 2 male patients which were negative for NS1. The presence of NS1 antigen along with IgM antibody were observed in 01 male and 01 female only as shown in fig-1.

The common clinical signs and symptoms among patients infected with DENV were also determined. Fever was predominantly observed in 100% of laboratory confirmed. Headache and fatigue was noted in 73% of the patients subsequently symptoms of the abdominal pain, vomiting, skin rashes were observed with a frequency of 60%, 54% and 52% respectively. Frequency of bleeding episodes was low and seen in only 5% of cases.

CMH Malir is a class A hospital having 500 beds capacity. In our study we have enrolled patient both from indoor and outdoor department, majority of the patients were from male medical ward (64/89, 72%) followed by outdoor (10/89, 11.2%) and female ward (9/89, 10.1%) each with few cases in ITC (4/89, 4.5%) and child ward (2/89, 2.3%) as shown in fig-2.

The distribution according to the various age groups was determined. Highest incidence of dengue infection was seen in the age group of 21-

40 years (63/89, 71%) followed by age groups of 40-60 years (16/89, 18%) whereas very low percentage was seen in extremes of ages (9/89, 10%) in 1-20 years and (1/89, 1.12%) above 60 years.

The majority of the case were reported in the month of August 22 (25%) followed by October 21 (23%) whereas no case of DENV infection was

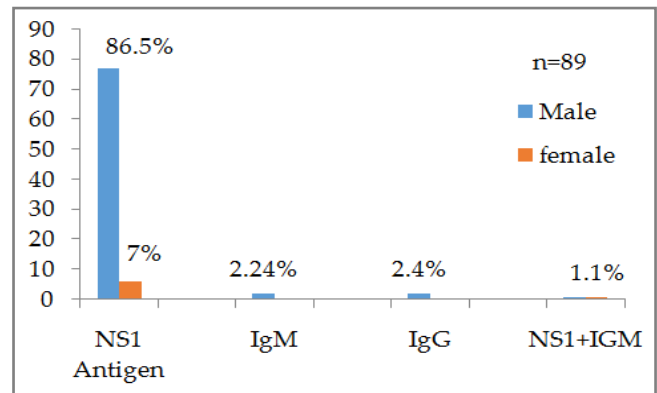


Figure-1: Laboratory marker of dengue virus infection.

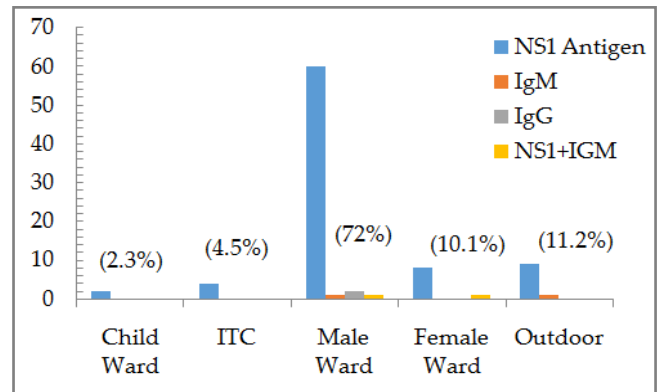


Figure-2: Dengue cases in various departments.

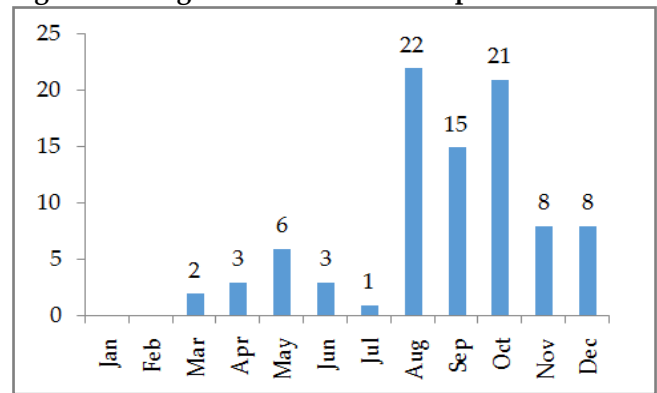


Figure-3: Month wise distribution DENV infection.

observed in the month of January and February as shown in fig-3.

DISCUSSION

Dengue virus infection is endemic in many tropics and subtropical regions of the world¹⁴. The global epidemiology of the disease has been changing since last 50 years and there is a surge in number of Dengue cases estimated 3.2 million cases with 2.35 million cases in America alone and around 1181 deaths per year as per recent data from WHO¹⁵, consequently it is important to establish its diagnosis as quickly as possible.

The prevalence of Dengue viral fever is global and the incidence varies from place to place. A review of epidemiological trends in south east Asia between 1980 and 2010 indicating increasing incidence of dengue infection, with annual average percentage change from 6% in Thailand, 10% in Vietnam, 12% in Indonesia, 18% in Malaysia and 24% in Philippines¹⁶. This cross sectional study of 1 year duration shows the burden of Dengue fever in patients who reported with acute febrile illness along with compatible clinical findings of Dengue, such cases were diagnosed by rapid diagnostic techniques use for an early diagnosis in different units of our tertiary care hospital.

During the study 993 suspected cases were examined and tested in which 89 (8.96%) were found to have lab confirmed DENV infection. In the studied populace fever was the common finding which was present in all, followed by headache, myalgia, abdominal pain, vomiting and skin rash whereas bleeding episodes were observed in only few subjects. These findings were consistent with a regional study from Saudi Arabia¹⁴, and a study from Pakistan which states that fever was seen in 100% followed by headache and fatigue in 73%, 7 except that the complications like bleeding tendency was much lower in our population compared to these studies, possible reason could be endemic dengue fever in our region compare to hyper endemic circulation which is responsible for Dengue hemorrhagic fever¹⁷.

In our study the dengue is seen mostly in population age between 21-40 years which is

in contrast to WHO report⁹, which states that children are more frequently effected during endemic circulation of virus (38%), the difference in the finding which was observed from our data is probably due to the military profession of most of the cases which lives in crowded conditions and this increases the potential for virus transmission.

Our study supports the previous finding that dengue cases occurs most frequently in post monsoon season as we observed the surge of dengue fever during August and October whereas no case was reported in the month of January and February in which the climatic conditions doesn't favor the breed of the vector¹⁵.

There are several studies in the literature assessing the efficacy of different diagnostic tests use for diagnosing DENV infection. Detection of DENV RNA by NAAT has high specificity but is more laborious and costly methodology though serology has lower specificity but is more accessible and economical so we adopted the second option for the diagnosis of dengue cases¹⁸. In the current study, NS1 antigen alone was more common positive finding in the majority of the cases. The results were in accordance with findings of Khan *et al* which evaluated the diagnosis of dengue during an outbreak in Swat in the year 2013 and stated that NS1 was positive in 3359 males and 1171 females out of total 6000 positive cases¹⁹. In primary DENV infection the sensitivity of NS1 detection can exceed 90% and antigenemia may last for several days even after resolution of fever whereas IgM can be detected after 4 days of onset of illness¹⁸, which was in accordance with our results as the samples were taken on the first day once patient presents with acute febrile illness and this can be the reason of more positive NS1 cases as compare to IgM.

Dengue NS1 rapid ICT method may be particularly useful providing early diagnosis because of faster turnaround time, another big advantage of the test include low cost which makes it a preferable choice as a diagnostic tool and overall it gives reliable results thus highly helpful in the

management of dengue cases in resource limited countries.

LIMITATIONS OF STUDY

There are few limitations in the present study. First serological test were carried out using rapid ICT method and RT-PCR of the positive cases was not done which is the reference standard method. Second serotyping was not carried out which is an important lab parameter to look for the strains of dengue virus prevalent in our region and thus helps in determining the endemic and hyper endemic circulation of virus.

In addition our study is a single centered study and cannot predict the actual incidence and epidemiological findings for whole region which requires multi centric studies.

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CONCLUSION

DENV infection can be early diagnosed by keeping high suspicion for dengue in patients who presents with acute febrile illness by using rapid diagnostic test with dengue specific NS1 antigen along with antibodies such as IgG and IgM.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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