EARLY DIAGNOSIS OF DENGUE VIRUS INFECTION IN CLINICALLY SUSPECTED CASES

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ABSTRACT

Objective: Comparison of real time reverse transcriptase polymerase chain reaction (RTPCR) and immunoglobulin M (IgM) capture enzyme linked immunosorbent assay (ELISA) for diagnosis of dengue virus infection in first week of illness in clinically suspected patients of dengue fever.

Study Design: Cross sectional study.

Place and Duration of Study: Department of haematology, Armed Forces Institute of Pathology (AFIP) Rawalpindi from Jan 2013 to Nov 2013.

Material and Methods: A cross sectional study including 68 clinically suspected patients of dengue fever according to the World Health Organization (WHO) criteria. IgM capture ELISA and RT PCR for dengue virus ribonucleic acid (RNA) was performed on samples collected from patients having fever for 1 to 7 days. These were divided into two groups. Patients in group 1 presented with fever of 4 days or less, patients in group 2 had fever of 5 to 7 days duration.

Results: In group 1, 72% of the patients were positive by RT PCR while 31% were positive by IgM capture ELISA. In group 2, 43% of the patients were positive by RT PCR while 97% were positive by ELISA.

Conclusion: RT PCR can be used for early detection of dengue virus infection in the first few days of fever while IgM ELISA is diagnostic afterwards.

Keywords: Dengue virus, IgM capture ELISA, RT PCR, WHO criteria.

INTRODUCTION

Dengue virus (DENV) is a Flavivirus transmitted by a mosquito bite (mostly by the *Aedes aegypti* species). It is a single stranded ribonucleic acid (RNA) virus which has four serotypes, DENV1-4. Dengue fever is dominant in Latin America, South-East Asia, and Pacific Asia; however, countries in South Asia, including Pakistan, India, and Bangladesh, have also reported an increasing number of outbreaks and brought significant impact on public heath[1-3]. The first confirmed epidemic of dengue virus in Pakistan occurred in 1994. Globalization accompanied with frequent international and domestic traveling also enhances the transmission probability of dengue[4,5]. A significant proportion of population in Pakistan has been affected by dengue virus, particularly during the outbreak in Lahore city in 2011.

Dengue infection causes fever which persists for 2 to 7 days. During the initial days of infection, blood complete picture reveals decreasing total leucocyte count (TLC) and platelet count. Laboratory diagnosis of dengue infection can be made by a number of tests such as virus isolation, Polymerase Chain Reaction (PCR) and serology (detection of immunoglobulin M (IgM) by enzyme linked immunosorbent assay (ELISA). Detection of IgM antibody against dengue virus by IgM capture ELISA is routinely used for the diagnosis of dengue fever. The initial days of infection which are characterised by fever are also the viraemic phase of the disease and dengue virus RNA can be detected during this period by Real Time Reverse Transcriptase Polymerase Chain Reaction (RT PCR). RT PCR has been shown to be more sensitive in the first 5 days of fever while serological tests are more

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Received: 26 Feb 2016; revised received: 10 May 2016; accepted: 12 May 2016

useful afterwards. Ahmed et al observed maximal sensitivity of real time RT-PCR assays for DENV detection on the second and third day of illness, respectively. In a study conducted by Khan et al the sensitivity of IgM ELISA was 83.5%[6]. In another study by Yong et al the sensitivity of RT PCR was 98.18%[7].

Specific diagnosis of dengue infection is difficult in the initial symptomatic period. IgM antibody against dengue virus is routinely detected for diagnosis of dengue infection in our setting. RT PCR was developed and used for the first time for the diagnosis of dengue fever at Armed Forces Institute of Pathology (AFIP) Rawalpindi.

The aim of this study was to compare RT PCR with IgM capture ELISA for early diagnosis of dengue virus infection. Early detection of dengue virus will not only help in early initiation of treatment but will also help in reducing morbidity and mortality associated with this disease.

MATERIAL AND METHODS

This cross sectional study was carried out at the department of Haematology AFIP, Rawalpindi during 2013. The sample size was calculated by WHO sample size calculator, keeping confidence level of 95%, anticipated population proportion 0.835, and absolute precision required 9%. The sampling technique was non probability purposive sampling. The sample size calculated was 68 patients. This study included a total of 68 suspected patients of dengue fever. All suspected patients of dengue fever irrespective of age and gender were included according to WHO criteria for probable dengue i.e. fever and 2 of the features (i) nausea and vomiting (ii) rash (iii) aches and pains (iv) leukopenia (TLC less than 4 X 10^9/L) (v) increase in haematocrit (Hct more than 0.5) concurrent with rapid decrease in platelet count (less than 150 X 10^9/L). All those patients with fever more than 7 days and malarial parasites on peripheral blood film were excluded from this study. The patients were divided into two groups according to duration of fever. In group 1, patients who had fever of 4 days or less duration were included while in group 2, patients who had fever of 5 to 7 days were included.

Two venous blood samples (2 to 3 ml each) were collected from the patients. One was taken in plain bottle for serum extraction and detection of IgM antibody against dengue virus by VIRCELL@ IgM capture ELISA. Second sample was collected in ethylene diamine tetra acetic acid (EDTA) for PCR after estimation of TLC, Hct and platelet count by Sysmex KX-21 automated hematology analyzer. For PCR, RNA extraction was done by QIAmp® viral RNA minikit 250 (QIAGEN) and complementary deoxyribonucleic acid (cDNA) was synthesized using reverse transcriptase and specific primer (DENV1-4:5'-TGATTCAACAGCACCATTCCAT) common for all dengue virus serotypes. RT-PCR was done by Taqman probe method using the following primers.

Table: Comparison of group 1 and 2.

	RT PCR	IgM ELISA
Group 1 (1-4 days fever) n=29	21 (72%)	9 (31%)
Group 2 (5-7 days fever) n=39	17 (43%)	38 (97%)

Forward primer

5'-AGAGACCAGAGATCCTGCTGTCTC

Reverse primer

5'-TGATTCAACAGCACCATTCCAT

Probe

5'FAM-AGCATCATTCCAGGCACAGAACGCC-TAMRA

These samples were run on ABI 7500 real time PCR machine. Data were entered and analyzed by using SPSS version 17.0. Mean and standard deviation were calculated for quantitative variables. Categorical variables were presented by frequency and percentages.

RESULTS

A total of 68 suspected dengue patients were included in the study. The mean and SD of the age of the patients was 34.1 ± 11.41 years (range:

5-60 years). Out of the 68 patients, 75% (51) were males and 25% (17) were females. All patients had fever at the time of sample collection and the duration of fever ranged from 1 to 7 days. Total leucocyte count was less than 4 x10^9/L in 36 (52%) patients while platelet count of less than 150 x10^9/L was seen in 46 (67.6%).

The number and percentage of patients found positive by RT PCR and IgM capture ELISA in both groups is shown in table, fig-1 and fig-2. In group I, out of 29 suspected patients 21 (72%) were positive by RT PCR and 9 (31%) were positive by IgM ELISA (7 patients was negative by both PCR and ELISA while one was positive by both). In group II (total 39 patients), 17 (43%) were positive by RT PCR and 38 (97%) were positive by ELISA (only one patient was negative by both tests). This shows that more cases were found positive by RT PCR in the group 1 as compared to IgM ELISA. Patients presenting on day 6 and 7 were all found to be positive by IgM ELISA showing that IgM ELISA is the method of choice for detection of dengue fever in patients having fever of more than 5 days.

DISCUSSION

In a study by Wang et al in 2010 dengue infection was detected by either MAC-ELISA or RT-PCR in 300 samples (93.75%), by MAC-ELISA in 229 samples (71.56%), and by RT-PCR in 162 samples (50.63%)[8]. In this study RT PCR was found positive in patients presenting with fever of 5 or lesser days while MAC-ELISA was positive after day 4 of illness. Our results matched the results of this study but with a very low sample size compared to this study.

Out of 68 suspected patients only 8 were found to be negative by both IgM capture ELISA and RT PCR. Chickengunya infection also presents with similar clinical features and thrombocytopenia but the difference is in the fact that TLC is always higher than 5 X10^9 in this infection[9]. As there is a lesser frequency of specific symptoms in early phase of illness therefore it is difficult to diagnose dengue based on clinical picture at this time. The frequency of maculopapular rash was 38% in our study compared to 11.2% to 41 in other studies[8,10]. In a study by Erum et al rash was found in 50% patients but in this study most patients had dengue hemorrhagic fever[5]. Retro orbital pain was found in 30% while in another study it was about 26%[10]. Therefore diagnosis cannot be made on the basis of these criteria but during epidemics

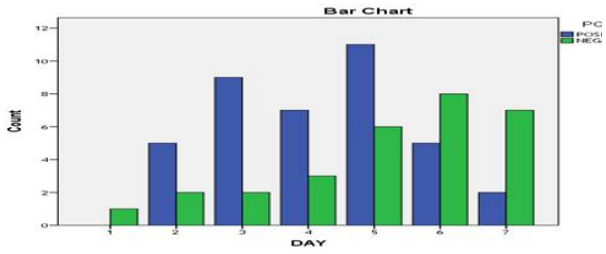

Figure-1: RT PCR positive cases according to days of fever.

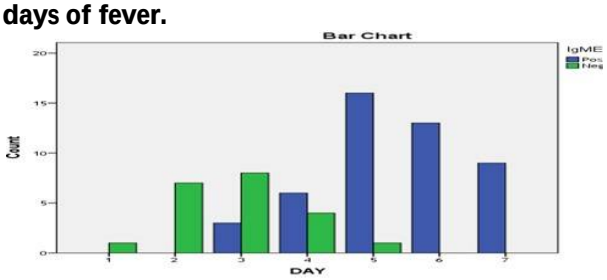

Figure-2: IgM capture ELISA positive cases according to day of fever.

they can be used to suspect dengue fever and start early treatment. The criteria for dengue diagnosis has high sensitivity (95%) when used during the first three days of illness but lacks specificity (40%)[9]. Laboratory parameters such as leucopenia and thrombocytopenia are also used in WHO criteria to suspect dengue fever. In our study leucopenia (TLC < 4X 10^9/L) was found in 52% patients while in a study by Erum et al it was 26%[5]. Leucopenia is seen in 40-48% of the patients in a study by Kuno et al[11]. Decrease in platelet count of < 100 × 10^9/l develops between day 3 to 7 of fever and then it returns to a normal level during second week[12]. In our study platelet count of <150 X10^9/L was found in 67.6% while patients having platelet count less than 100 X 10^9/L were 50%. Studies by Ayyub et al, Ali et al and Nauskeri et al showed that platelet count of less than 100 was found in 77-86% of patients[12-13]. In these studies most patients had fever of more

than 3 to four days which can be the reason for more patients having thrombocytopenia.

CONCLUSION

RT PCR can be used for early detection of dengue virus infection in the first few days of fever while IgM ELISA is diagnostic afterwards.

CONFLICT OF INTEREST

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

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