Original Article

Categorisation of Dengue based on duration of fever and serological markers in a tertiary care hospital

[1]Dr.P.Malini, [2]Dr.M.Sumathi , [3]Dr.T.Ravikumar

[1]Assistant Professor of Microbiology, GMC &ESI Hospital

[2]Associate Professor of Pathology, GMC&ESI Hospital

[3]Professor of Medicine, GMC&ESI Hospital

Corresponding author: Dr.M. Sumathi , Associate Professor of Pathology ,GMC&ESI Hospital

ABSTRACT:

Introduction: Dengue is considered as one of the world's major emerging tropical diseases. Serological diagnostic methods for NS1,IgM and IgG detection has been the mainstay of diagnosis for a long time.

Aim and objectives: Categorisation of Dengue based on Fever and NS1,IgM,IgG in a tertiary care hospital.

Materials and methods: Blood was drawn from 350 suspected cases and subjected to ELISA.

Results: Out of 350 samples 107 were positive by ELISA.Out of 107, 27 - NS1 positive ,8 - NS1 &IgM, 9 -IgM alone, 4 - IgM&IgG ratio >1,NS1 &IgG -1,NS1,IgM&IgG-14,IgM&IgG ratio <1-38 , IgG alone positive with thrombocytopenia -6.

Conclusion: All the dengue positive cases need to be categorised based on serological markers before treatment which can reduce the mortality and morbidity rate. NS1 antigen ELISA to be added with routine antibody testing protocols for early detection and management of cases .

Key words: NS1-Nonstructural protein 1, PD-primary dengue ,SD- secondary dengue.

Introduction:

According to WHO, Dengue fever or Dengue haemorrhagic fever is considered as the second most important tropical disease next to malaria[1].Dengue in recent years has become a major international health problem. Annually there are 100 million new dengue viral infections reported worldwide with 5 lakh cases of Dengue haemorrhagicfever(DHF) and Dengue shock syndrome(DSS). There are around 30000 deaths every year which is mostly among children[2,3,4]. DHF/DSS is known as one of the leading causes of mortality and morbidity among school going children in Tropical and Subtropical countries[5].

Dengue virus is a single stranded RNA virus with four serotypes. They are DEN1, DEN2, and DEN3&DEN4. All the four serotypes are endemic in India[6,7].Infection with one serotype of Dengue does not confer cross protection against the other serotype. On subsequent infection it may lead to serious forms of disease like Dengue haemorrhagic fever and Dengue shock syndrome through immuno–pathological enhancement[8,7]. Serological diagnosis of Dengue has many advantages like more flexibility, wide availability of reagents, low cost and requirementof less equipments[7.] One of the definite methods to diagnose early Dengue infection is to detect specific antigen which directly correlate with underlying viremia and pathogenesis of infection[10,9] In view of the increased occurrence of Dengue and its complications, the study is undertaken to look for categorization of Dengue among patients attending tertiary care hospital based on serological markers.

Materials and Methods:

Prospective study conducted in the department of Microbiology, GMC&ESI Hospital for a period of of 3 years.

Inclusion criteria: patients suffering from fever of 1-12 days with symptoms like myalgia, arthralgia,headache ,rash,anorexia,nausea,vomiting and abdominal pain.

Exclusion criteria: Patients with non specificfever, urinary tract infection, pneumonia lung abscess.

Methodology: 5mlof venous blood was drawn from 350 suspected patients after informed consent. Serum separated at 1500 rpm for 10 minutes and subjected to NS1antigen, IgM & IgG antibody ELISA.

Results:

Statistical analysis of this study was done using SPSS version 17. P value obtained from pearson chi-square test. Seropositives were categorized as primary 48(44.9%) and secondary dengue 59(9.4%) with ratio 0.8:1 based on fever duration (1-5 days, 6-10&>10) and serological markers like NS1 ,IgM&IgG.

Table 1

Serological Categorisation of Dengue positives based on ELISA

Category	No. of Positive Cases	Percentage
Primary Dengue	48	44.9%
Secondary Dengue	59	55.1%

Table 2

Primary dengue based on duration of fever &serological markers

Fever duration	NS1 positive	NS1&IgM Positive	IgM alone Positive	IgM&IgG+ve withRatio>1
1-5 Days (37)	**27 (72.9%)**	**8 (21.6%)**	**2 (5.4%)**	**0 (0%)**
6–10 Days (10)	**0 (0.0%)**	**0 (0.0%)**	**7 (70%)**	**3 (30%)**
> 10 days (1)	**0 (0.0%)**	**0 (0.0%)**	**0 (0.0%)**	**1 (100%)**
Total (48)	**27 (56.25%)**	**8 (16.6%)**	**9 (18.75%)**	**4 (8.33%)**

Chi-square test=47.07, P value<0.001(significant)

www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858

Table 3

Secondary Dengue based on duration of fever and serological markers

Fever duration	IgG+thrombocytopenia	NS1 &IgG Positive	NS1,IgM&IgG Positive	IgM&IgG+ve with Ratio <1
1-5 Days (23)	5 (21.7%)	1 (4.3%)	11 (47.8%)	6 (26.08%)
6 – 10 Days (26)	1 (3.8%)	0 (0.0%)	3 (11.5%)	22 (84.6%)
> 10 Days (10)	0 (0.0%)	0 (0.0%)	0 (0.0%)	10 (100%)
Total (59)	6 (10.1%)	1 (1.6%)	14 (23.7%)	38 (64.4%)

Chi-square test= 25.12, P value<0.001(significant)

Discussion:

In this study the incidence of Primary dengue was 48(44.8%) & secondary dengue was 59(55.1%) in the proportion of 0.8:1. In a study by **Jonathan et al 2010**[11]SD was more than PD .**Neerajaet al**[12] study showed 72.5% of SD.

During the primary infection NS1 gets secreted out from virus infected cells which indicates underlying viremia & pathogenesis of infection. As the antibody titre is very low in primary dengue ,NS1 is detected morein acute period. Hence it can be used as standalone test for PD in cases where IgM is not detectable &PCR not available.

During the acute period **of 1-5 days in primary dengue** , NS1 detection (72.9%) had higher positivity followed by NS1 &IgM(21.6%) and IgM alone positive of 2(5.4%). As IgM antibodies tends to appear after 4-5 days of infection and as many patients in this study presented within 5 days, the sensitivity of NS1 &IgM ,IgM alone positive in primary dengue were less when compared to NS1 alone positivity . Hence when IgM ELISA is negative in the acute period, NS1 ELISA should be done for diagnosis which significantly improves the detection rate without the requirement of paired serum to demonstrate the rise in titre of antibodies. In a study by **Chakravarti A, Kumar A, Malik S**[13]2011New Delhi showed NS1 ELISA positivity rate of 65.9% and MAC-ELISA showed 60.2% positivity in the acute period. **Veasnaduonget al**[9] study also showed NS1 positivity to be higher in first 3 days. **Minipritamsinghet al**[14] and **Chua KB et al 2011**[15]studies revealed that NS1 antigen capture ELISA was more sensitive than RT-PCR and viral isolation.

During the early convalescent period **of 6-10 days in primary dengue**, IgM showed higher positivity (70%) than other serological markers due to the appearance of IgM antibodies after 4th day of illness and is the first antibody to appear following Dengue infection. IgM antibody gradually increases towards end of first week and it persists for 3 months thereafter. NS1 positivity was less in this period as they tend to be in complex with IgM antibody. IgM and IgG combination also showed less positivity (30%) as anti dengueIgG appears later and detectable only at low titer during this period . In a study by **S Datta and Wattal**[16]IgM detection by MAC ELISA showed 93.6% positivity in early convalescent period.

www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858

In more than **10 days duration**, IgM&IgG positive with ratio > 1 showed higher positivity (100%) as IgG antibodies would have appeared during this period which increases further in the proceeding weeks and persists lifelong thereafter.

In this study, in secondary dengue, IgM and IgG positive with ratio <1 showed higher positivity (64.4%) than other parameters followed by NS1, IgM and IgG combination (23.7%). As the antibody titres rise extremely rapidly, IgM and IgG are detected more in secondary dengue.

During the acute period of **1-5 days in secondary dengue** NS1, IgM and IgG combination showed higher positivity11(47.8%) than IgM&IgG combination 6 (26.08%),NS1&IgG 1(4.3%) and IgG alone positive with thrombocytopenia 5(21.7%).The NS1 antigen has reduced sensitivity because it is sequestered in immune complexes and that target epitopes are not accessible to the plate bound mAb in NS1 ELISA. High levels of IgG are detectable even in acute phase and rise dramatically over the proceeding 2 weeks whereas IgM levels tend to be significantly lowered in secondary infection.

In the early convalescent period **of 6-10 days in secondary dengue**IgM&IgG positive with ratio<1 22 (84.6%) showed higher positivity as IgM to second infection appears during this period. NS1, IgM&IgG showed positivity of 3(11.5%), IgG alone positive with thrombocytopenia 1(3.8%). As IgG is present in both current and past infections, presence of IgG alone does not indicate active infection. Hence it should be combined with platelet count. Only if the platelet count is lowered it indicates current infection. If it is normal, it shows past infection. In more than 10 days duration of secondary dengue IgM&IgG positive with ratio <1showed higher positivity (100%) which is due to the presence of IgM&IgG antibodies to the second infection.

Conclusion:

For diagnosis of Dengue infection irrespective of duration of fever all the three (i.e) NS1, Dengue IgM and IgG byImmunocapture ELISA must be done .Serological differentiation of primary and secondary dengue can be arrived as per the results of the study as shown below:

NS1	**IgM**	**IgG**	**Category**
+	-	-	Primary dengue
+	+	-	Primary dengue
-	+	-	Primary dengue
-	+	+	Primary dengue(IgM/IgG ratio>1)
-	-	+	Past infection-(Plateletcount&Total count normal).
-	-	+	Secondary dengue-(Platelet/Total count low)
+	-	+	Secondary dengue
+	+	+	Secondary dengue
-	+	+	Secondary dengue-(IgM/IgG ratio <1)

www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858

In conclusion, based upon the higher positivity of the NS1 ELISA both in primary and secondary dengue, this study gains public health importance because it suggests that NS1 antigen should be included along with routinely used antibody testing protocols for early detection and management of cases.

References:

1. Mandell's, Douglas and Bennett's principle and practice of infectious disease.7th edition.Vol.2
2. Seema and S.K.Jain Molecular mechanism of pathogenesis of Dengue virus: Entry and fusion with target cell. Indian journal of clinical biochemistry, 2005, 20(2) 92-103
3. Abmshahidulalamet al Bangladesh J child health 2009; 33:2:p.no 55-58
4. WHO (2009) Dengue: guidelines for dengue diagnosis, treatment, prevention and control, 3 rd Edition.
5. SukathidaUbol ,Promsinmusrinoul,Jeerayutchaijaruwanich et al Differences in Global gene expression in peripheral blood mononuclear cells indicate a significant role of the innate responses in progression of Dengue fever but not Dengue haemorrhagic fever .The Journal of infectious Diseases 2008 ;197 :1459-67
6. R D Kulkarni,SSPatil,GS A jantha et al Association of platelet count and serological markers of dengue infection- importance of NS1 antigen Indian journal of Medical Microbiology2011 29(4):359-362
7. Manson's tropical disease 21st edition .S.Nimmannitya chapter 42 Dengue and dengue haemorrhagic fever.
8. Tamilarasukadhiravan et al Association of intracellular Th1-Th2 balance in CD4+Tcells and MIP-1α in CD8+ T cells with disease severity in adults with Dengue.
9. Veasna Duong et al Clinical and virological factors influencing the performance of a NS1 Antigen capture Assay and potential use as a marker of Dengue Disease severity PLOS Neglected Tropical diseases. 2011 5(7*).*
10. Vu Ty Hang et al Diagnostic accuracy of NS1 ELISA and Lateral flow Rapid Tests for Dengue sensitivity, specificity and relationship to Viremia and Antibody response. PLOS Neglected Tropical diseases. 2009 3(1)
11. Jonathan et alprofile of paediatric patients with denue fever /Dengue haemorrhagic fever over a five year period (2000-2004) PIDSP journal 2010;11:1
12. NeerajaM,LakshmiV,Teja VD et al Serodiagnosisog dengue virus infection in patients presenting to a tertiary care hospitalBioline international official site May 2006.
13. ChakravartiA,KumarA,Malik S Detection of dengue infection by combining the use of an NS1 antigen based assay with antibody detection. Southeast Asian J trop Med Public Health ,2011 Mar;42(2):297-302
14. Minipritamsingh et al NS1 antigen as an early diagnostic marker in dengue: report from India. Diagnostic Microbiology &infectious diseases.2010;68:1:p.50-54
15. Chua KB, Mustafa B, Abdul Wahab et al A comparative evaluation of Dengue diagnostic tests based on single acute serum samples for laboratory Confirmation of acute dengue. Malaysian journal of pathology 2011jun;33(1):13-20
16. S DattaC.Wattal Dengue NS1 antigen detection : A useful tool in early diagnosis of dengue virus infection Indian Journal of Medical Microbiology (2 010)28(2):107-10