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Original Research Paper

PROPORTION OF PRIMARY AND SECONDARY DENGUE INFECTION AMONG SEROLOGICALLY POSITIVE DENGUE FEVER CASES ADMITTED IN A TERTIARY CARE CENTRE IN KERALA

Authors:

Dr. Ria Catherine Vincent[1], Dr. Joana Mary Magdaline[2], Dr. Jacob K. Jacob[3]

[1]Senior Resident, Department of Microbiology, Government Medical College Ernakulam, PIN – 683503, Kerala, India
[3]HOD, Department of Medicine, Government Medical College Ernakulam, PIN – 683503, Kerala, India

Corresponding Author:
Dr. Joana Mary Magdaline, HOD, Department of Microbiology, Government Medical College Ernakulam, PIN- 683503, Kerala, India

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ABSTRACT:

Introduction: Dengue fever is the most rapidly spreading mosquito borne viral disease in the world. In the recent decades global incidence of dengue has significantly increased. India is endemic for dengue so is Kerala state. Increased population growth, urbanisation, lack of efficient mosquito control programme contribute to its spread. Secondary dengue infection is more prone to develop complications such as DHF and DSS. **Objectives:** The present study was conducted to estimate the proportion of primary and secondary dengue infection among serologically positive dengue fever cases admitted in Government medical college Ernakulam. **Methods:** This descriptive cross-sectional study conducted at Government Medical College Ernakulam included 450 clinically suspected cases of dengue whose blood samples were received in Microbiology lab for testing. Clinical history of patients was taken from case records. NS1, IgM, and IgG ELISA were performed with serum from each sample according to manufacturer's instructions. To detect the proportion of primary and secondary dengue cases, IgM to IgG optical density ratio was calculated in those samples positive for NS1/IgM/IgG. IgM/IgG optical density ratio more than 1.2 indicated primary dengue infection and IgM/IgG optical density ratio less than 1.2 indicated secondary dengue infection. The data was entered in the excel spread sheet as per proforma and was analysed using SPSS software. Chi square test was used for analysis of study variables. The level of significance was taken as p value < 0.05. **Results:** Out of the 450 samples tested, 147(32.66%) were found to be positive for dengue. Secondary dengue infection was more (56.4%). **Conclusion:** The proportion of dengue positivity was comparable to many other studies done in India. Secondary dengue infection was more in our study which can be attributed to hyperendemicity of dengue in Kerala.

Keywords: Primary dengue, Secondary dengue, Dengue hemorrhagic fever, Dengue shock syndrome

INTRODUCTION:

Dengue fever (DF) is one of the most important infectious diseases among the tropical and subtropical regions of the world. It poses significant economic and disease burden in endemic countries (1). Prior to 1970, DF was prevalent only in 9 countries. Since then there has been 4 fold increase in number of cases, still increasing (2). The global incidence of dengue hemorrhagic fever (DHF)/ dengue shock syndrome (DSS) has increased more than 500-fold affecting more than 100 countries (3). Unchecked population growth and urbanisation, uncontrolled population movement, substandard housing, poor water storage facilities and improper waste management systems, absence of effective mosquito control programme, increased air travel and deterioration of public health facilities are the contributing factors (4). The most effective way to prevent disease rely on control of the vector mosquito and prevention of mosquito bite (1). In India dengue is a major public health problem and a leading cause of hospitalisation and death among children due to hyperendemicity of all four serotypes circulating in urban areas, and with spread to rural areas (5). Kerala is one of the worst affected states by DF in India (6). The first outbreak of dengue in Kerala was reported in Kottayam district in 1997 and many outbreaks occured in subsequent years (6). In 2001, 4 districts of Kerala

famous for rubber plantations, Kottayam, Idukki, Ernakulam and Thiruvananthapuram, had outbreaks (6). Since 2007, the number of cases has been increasing (7). The highest number of cases occurred in the outbreak in the year 2017 with 165 deaths (7). Dengue virus belongs to family Flaviviridae and has 5 serotypes. It is an enveloped virus with a single stranded RNA genome (3)(8). Dengue transmission occurs usually during the rainy season when the temperature and humidity are conducive for vector population breeding in secondary habitats as well as for longer mosquito survival (9). Dengue virus transmission to humans occur by the bites of the vector infected female mosquitoes, primarily Aedes aegypti which is a day biting mosquito (10)(11). Aedes aegypti is seen more in and associated to human inhabitation. It is more restricted to warm and urban regions (12). Others are Aedes albopictus, Aedes polynesiensis etc. Rarely vertical transmission from mother to baby have been documented (10). Primary dengue infection is usually benign and might be asymptomatic. Secondary infection with a different serotype cause severe infection leading to complication such as DHF, DSS (4). Typical DHF is characterized by four major clinical manifestations: high fever, haemorrhagic phenomena, hepatomegaly and circulatory failure (13). Moderate to marked thrombocytopenia with concurrent haemoconcentration is a distinctive finding (13). A rapid, weak pulse with narrow pulse pressure (20 mm of Hg), regardless of pressure levels, or hypotension with cold, clammy skin and restlessness is seen in dengue shock syndrome (13). Patients may pass into a stage of profound shock with pulse impalpable (13). Most dengue infections are subclinical (10). Most DHF occurs in secondary infections (10). Almost all the patients are viremic during clinical presentation with fever. Clearance of virus from blood occurs within days after defervescence (10). During primary dengue infection, dengue specific IgM appears first, followed by IgG (14). The antibody response starts by 5th day and IgM and IgG antibodies are of diagnostic value (9). IgM antibodies start to appear by 3 to 5th day after the onset of illness, rise quickly by about 2 weeks and decline to undetectable levels by 2 to 3 months (9). IgG antibodies are detectable at low levels by the end of first week, followed by an increase in levels and persistence for a longer period (9). In the initial phase of secondary dengue infection IgG antibody titre rise rapidly and IgM antibody titres are low (9). Hence IgM/IgG optical density ratio is used for differentiation of secondary from primary dengue (9). IgM /IgG ratio had good sensitivity, specificity and accuracy in differentiating primary and secondary dengue (15). Following dengue infection there is production of both neutralising and non-neutralising antibodies (16). Neutralising antibodies provide protection against infective serotype which last lifelong (16). Non-neutralising antibodies persist lifelong and they are produced against other serotypes, not the infecting serotype (16). Non-neutralising antibodies contribute to viral replication and disease severity in secondary infection with a heterotypic dengue virus (16). This phenomenon is known as antibody dependent enhancement (ADE) (16). Lab diagnosis of dengue include direct methods such as virus isolation, viral nucleic acid detection by PCR, NS1 antigen detection and indirect methods such as immunological tests for detection of IgM and IgG antibody (9). There are also certain nonspecific tests such as total leucocyte count, platelet count, hematocrit, liver enzymes, serum albumin level (9). NS1 antigen detection and PCR are considered to be better tests to diagnose dengue in early phase (15). Since IgM appears after 5th day, antibody based serological tests are usually negative during first 5 days and later IgM detection is better (9)(15). IgG antibodies rapidly rise during the initial phase and persists for a long period whereas IgM antibodies are of lower titre in secondary cases (9). So IgM/IgG optical density ratio is used for differentiation of secondary from primary dengue (9). If the ratio is more than 1.2 it is primary dengue and if less than 1.2 it is secondary dengue (9). The crucial point in dengue treatment is the identification of the phase in which the patient is in at the time of presentation and providing the correct management (10). Fatalities occurring are mostly because of late presentation, or overzealous fluid management (10). Management of DF is symptomatic and supportive (17). Bed rest is an essential component (17). Patient has to be monitored carefully for signs of complication (17). Pulse, blood pressure, haematocrit, platelet count need close monitoring daily (17). According to the condition intravenous fluid therapy can be started (17). If there is thrombocytopenia platelet transfusion need to be given (17). If there decrease in haematocrit and if bleeding doesn't stop blood transfusion need to be given (17). Prevention of dengue include general methods such as vector control which include anti-larval measures, anti-adult measures, personal protective equipment and specific methods such as administration of vaccine (18)(19). Different vaccines for dengue include live attenuated vaccine, recombinant chimeric vaccine, DNA vaccines, recombinant protein vaccine and subunit vaccine (20)(21). Dengvaxia is a recombinant chimeric dengue vaccine licensed to Sanofi Pasteur (21). Dengvaxia has been approved for use in 20 countries including European Union and United States (22). Efficacy of the vaccine is variable (23)(24).

AIMS AND OBJECTIVES:

The present study was conducted to estimate the proportion of primary and secondary dengue infection among serologically positive dengue fever cases admitted in a tertiary care centre in Kerala.

RELEVANCE OF THE STUDY:

Kerala state is endemic for dengue viral infection (25). During 2017 outbreak Kerala reported highest number of dengue cases and deaths due to dengue (26). There are very few studies on dengue in central Kerala especially on serologically diagnosed dengue cases. Dengue infection gives lifelong protection against the causative serotype (27). Secondary infection with a different dengue virus serotype after the primary infection can result in complications (27). There is no definite treatment for dengue, hence early diagnosis and supportive treatment is necessary to prevent complications (28). It is also necessary to differentiate between primary and secondary dengue infections so that watchful expectancy can be maintained regarding development of complications and prompt measures can be taken to reduce the severity (28). Hence this study is done to find the proportion of primary and secondary dengue infection among serologically positive dengue fever patients. All 4 serotypes are prevalent in Kerala which increase the risk of complications such as DHF and DSS which adds to the relevance of the study (28).

MATERIALS AND METHODS:

Study design: Descriptive cross-sectional study

Study setting: Department of Microbiology, Departments of Medicine and Paediatrics of Government Medical College Ernakulam

Study period: 1 year (January 2019 – December 2019)

Sample size was calculated as 450.

Inclusion criteria: Blood samples received in Microbiology lab from all patients with clinically suspected dengue.

Exclusion criteria: Haemolysed and macroscopically lipemic blood sample were excluded.

Study procedure: NS1 antigen detection, IgM and IgG antibody detection by ELISA were performed in all the samples from clinically suspected dengue patients as per manufacturer's instructions. Serum was used for performing all the tests. IgM to IgG optical density ratio was calculated for all samples positive for NS1/IgM/IgG alone and in combinations.

Dengue NS1 ELISA: was performed using kit by CTK diagnostics (LOT NO: E1107Q2D00, Expiry date: April 2020. NS1 antigen ELISA is a solid phase enzyme linked immunosorbent assay. It is based on the principle of antibody sandwich technique for the detection of dengue NS1 antigen in human serum or plasma.

Specimen OD ratio < 1 – interpreteted as negative for dengue NS1 antigen

Specimen OD ratio ≥ 1- interpreted as positive for dengue NS1 antigen

Dengue IgM ELISA: was performed using kit by J. Mitra & Co. (LOT NO: EDMO 10419, Expiry date: September 2020). Dengue IgM Microlisa test is based on "MAC Capture ELISA". Microtiter wells are coated with antihuman IgM antibodies. If specimen contain dengue IgM antibody, it will bind to the anti-human IgM antibodies adsorbed onto the surface of the wells.

a. Dengue IgM Units < 9 - interpreted as negative for dengue IgM antibodies.

b. Dengue IgM Units > 11 - interpreted the as positive for dengue IgM antibodies

c. Dengue IgM Units between 9 – 11: interpreted as equivocal for dengue IgM antibodies. Test was repeated.

Dengue IgG ELISA: was performed using kit by J. Mitra & Co. (LOT NO: EDGO 10318, Expiry date: August 2019). Dengue IgG ELISA is an enzyme immunoassay based on the principle of "GAC-Capture ELISA". Microtiter wells are coated with antihuman IgG antibody. If the specimen contains dengue IgG antibody, it will bind to the anti-human IgG antibodies adsorbed onto the surface of the wells.

a. Dengue IgG Units < 9: interpreted as negative for dengue IgG antibodies.

b. Dengue IgG Units > 11: interpreted as positive for dengue IgG antibodies.

c. Dengue IgG Units between 9 - 11: interpreted as equivocal for dengue IgG antibodies. Test was repeated.

IgM/IgG ratio: Optical density ratio of IgM to IgG was calculated.

If IgM/IgG optical density ratio > 1.2 – interpreted as primary dengue (9).

If IgM/IgG optical density ratio <1.2 – interpreted as secondary dengue (9).

Data collection and entry: Clinical details were collected from clinical case records and entered in the proforma along with results of the tests done. The type of dengue case whether primary or secondary was also noted. All these data were numerically coded and entered in Microsoft Excel Spreadsheet.

Data analysis: The data entered in the MS-Excel spreadsheet was analysed using Statistical Package for Social Sciences (SPSS) software 16.0. Categorical variables were expressed as proportion. Association

between variables was tested using Chi – square test and Fisher's exact test. The level of statistical significance was taken as p value < 0.05.

RESULTS:

Total number of samples tested were 450. Of these 147(32.66%) samples were positive by NS1/IgM/IgG ELISA. Among the dengue positive cases proportion of primary dengue was 43.53% and secondary dengue was 56.46%.

Figure 1: Distribution of dengue patients as primary and secondary dengue

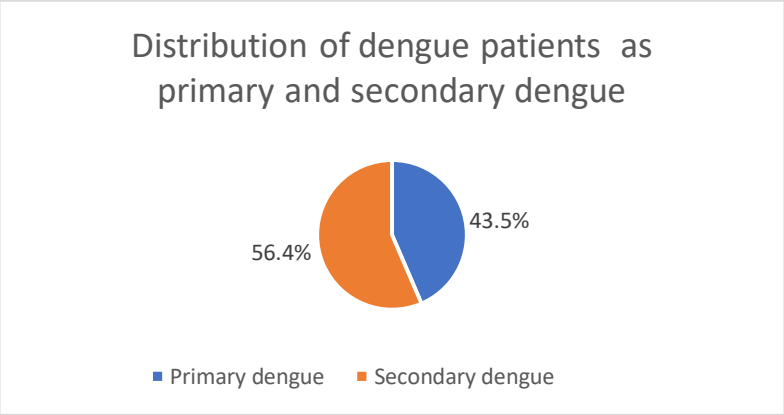

Table 1: Distribution of dengue patients by serological status

Serological status	No. Of dengue patients (n=147)
NS1	61(41.4%)
IgM	101(68.7%)
IgG	77(52.3%)

Around 50% were positive for more than one serological parameter.

Table 2: Distribution of dengue patients as DF, DHF, DSS

DF/DHF/DSS	Number
Dengue fever	128(87%)
Dengue hemorrhagic fever	15(10.2%)
Dengue shock syndrome	4(2.7%)
Total	147

In our study majority (87%) presented as dengue fever. Only 4.6% of primary dengue presented with DHF. All patients with DSS had secondary dengue infection.

Table 3: Distribution of dengue patients based on thrombocytopenia

Primary / secondary dengue	Severe thrombocytopenia	Moderate thrombocytopenia	Mild thrombocytopenia	Total
Primary dengue(n=64)	5(7.8%)	4(6.2%)	15(23.4%)	24
Secondary dengue(n=83)	22(26.5%)	12(14.4%)	30(36.1%)	64
	27	16	45	88

Thrombocytopenia was seen more in secondary dengue cases than primary. Significant association was seen between secondary dengue and decreased platelet count (p value = 0.000014). In our study among cases with abnormal leucocyte count both primary and secondary dengue showed leucopenia than leucocytosis.

Table 4: Distribution of dengue patients based on raised haematocrit

	Raised haematocrit
Primary dengue(n=64)	4(6.2%)
Secondary dengue(n=83)	17(20.4%)
Total	21

In our study although in majority of dengue positive cases haematocrit was normal, percentage of secondary dengue cases which showed raised haematocrit was remarkably higher than primary cases. By Fisher's exact test significant association was seen between secondary dengue and altered haematocrit. (p value = 0.0171).

Table 5: Distribution of dengue patients based on raised ALT (Alanine aminotransferase)

Liver enzymes	Primary dengue	Secondary dengue
Alanine aminotransferase raised(n=22)	4(18.8%)	18(81.8%)

In our study among the 22 patients with raised ALT , 81.8% had secondary dengue infection. Statistically significant association was seen between secondary dengue and raised ALT by Fisher's exact test.(p value = 0.0102).

Table 6: Distribution of dengue patients based on raised AST (Aspartate aminotransferase)

Liver enzyme	Primary dengue	Secondary dengue
Aspartate aminotransferase raised (n=24)	4(16.6%)	20(83.3%)

Raised AST was seen significantly higher among secondary dengue patients than primary in our study. By Fisher's exact test significant association was seen between secondary dengue and raised AST level(p value= 0.0035)

Table 7: Distribution of dengue patients based on serum albumin level

Serum albumin	Primary dengue	Secondary dengue
Decreased	0(0%)	12(14.4%)
Normal	64(100%)	71(85.5%)
Total	64	83

Decrease of serum albumin was seen more in secondary dengue in our study. Statistically significant association was seen between secondary dengue and decreased albumin level (p value = 0.0012).

Table 8: Distribution of dengue patients based on outcome

Outcome	Dengue patients (n=147)
Survived	147(100%)
Expired	0(0%)

In spite of complications of dengue fever namely DHF, DSS in 19 patients there was no case fatality in our study.

DISCUSSION:

The present study was conducted in the Department of Microbiology, Department of Medicine and Paediatrics of Government Medical College Ernakulam over a period of one year starting from January 2019 to December 2019 to estimate the proportion of primary and secondary dengue infection among the serologically positive dengue fever cases. Total number of samples tested were 450. Of these 147(32.66%) samples were positive by NS1/IgM/IgG ELISA. In our study primary dengue was found to be 43.5% and secondary dengue to be more ie; 56.4%. This can be attributed to hyperendemicity of dengue in Kerala state. Similar finding was seen in studies conducted in Thiruvananthapuram in 2008, Northern India in 2015, Karnataka in 2017 (29)(30)(31). But a study done in Mangalore in 2013 showed a lower prevalence of secondary dengue (21.66%) (32). In our study complications such as DHF and DSS were more common in secondary dengue and only 4.6% of primary dengue presented with DHF. All patients with dengue shock syndrome had secondary dengue infection in our study. Similar observation was seen in studies done in Thiruvananthapuram in 2008, Perambalur in 2013 and Northern India in 2015 (29)(30)(33). Thrombocytopenia was seen more in secondary dengue cases than primary in our study. By Chi square test, significant association was seen between secondary dengue and decreased platelet count (p value=0.000014) in our study. This was in concordance with studies done in Rawalpindi in 2013, Northern India in 2015, Dehradun in 2018 (30)(34)(35). Contrasting finding was seen in a study done in Varanasi in 2016 which showed thrombocytopenia more among primary dengue patients (36). Usually in dengue leucopenia is more common than leucocytosis. In our study among cases with abnormal leucocyte count both primary and secondary dengue showed leucopenia than leucocytosis. There were not much studies done for association of dengue status with altered leucocyte count. Raised haematocrit leading to haemo-concentration is a feature of dengue. In our study although in majority of dengue positive cases haematocrit was normal, percentage of secondary dengue cases which showed raised haematocrit was remarkably higher than primary cases. By Fisher's exact test significant association was seen between secondary dengue and altered haematocrit. (p value = 0.0171). Similar finding was seen in a study done in Africa in 2016 (37). Contrasting finding was seen in a study done in Perambalur in 2013 which showed that majority of dengue patients didn't have changes in haematocrit (33). In our study raised ALT and AST was found to be significantly higher among secondary dengue patients which was statistically proved. Similar observation was seen in a study conducted in Africa in 2015, Tiruvalla in 2016 (37)(38). Contrasting finding was seen in a study done in Dehradun in 2018 which showed raised AST more in primary dengue cases (35). Hypoalbuminemia due to plasma leakage is a feature of complicated dengue. In our study decrease of serum albumin was seen more in secondary dengue which was statistically significant (p value=0.012). There were not much studies showing association between hypoalbuminemia and secondary dengue. Inspite of complication of dengue fever namely DHF, DSS in 19 patients there was no case fatality in our study. Although there is no specific treatment for dengue 100% survival of the dengue positive patients in our study can be attributed to multiple factors such as early detection, aggressive resuscitation and timely and adequate supportive treatment. This was in concordance with studies done in Haryana in 2010, Tiruvalla in 2016, North Kerala in 2017 (38)(39)(40).

CONCLUSION:

A total of 450 clinically suspected dengue patients admitted in Medicine and Paediatrics wards of Government Medical College Ernakulam were included in the study from January 2019 to December 2019. Among the total samples 147(32.66%) tested positive for dengue by NS1/IgM/IgG ELISA. Around 50% patients were positive for more than one serological parameter. Among the dengue positives proportion of primary dengue was 64(43.53%) and that of secondary dengue was 83(56.46%). 19 cases had complication namely DHF, DSS. Alteration in lab parameters such as

thrombocytopenia, raised haematocrit, elevated liver enzymes, hypoalbuminemia showed statistically significant association with secondary dengue. Case fatality was nil as observed in our study.

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