ORIGINAL RESEARCH

Evaluation of Antibodies Titre in Diagnosing Cases of Dengue Fever: An Institutional Based Study

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ABSTRACT

Background: Dengue infection is the most prevalent arthropod-borne viral disease in subtropical and tropical regions of the world. The present study was conducted to assess the effectiveness of antibodies in diagnosing cases of dengue fever. **Materials & Methods:** This study was conducted on 142 patients. For detection of NS1 antigen, IgG and IgM antibody rapid immune chromatography method was used. **Results:**Out of 142 patients, males were 82 and females were 60. Common clinical features were headache in 76, vomiting in 89, abdominal pain in 94, myalgia in 52, hepatomegaly in 48 and bleeding in 36 cases. The difference was significant (P<0.05). Among serum samples, 90% found positive for NS1, IgG (4%), IgM (2%). 1.3% for both NS1 and IgG, 1.2% for NS1 and IgM and0.50% for NS1, IgM and IgG. The difference was significant (P<0.05). **Conclusion:** Dengue fever is common nowadays. It was found that NS1, IgG and IgM are diagnostic marker for dengue fever.

Key words: Dengue fever, NS1, IgG, IgM.

INTRODUCTION

Dengue infection is the most prevalent arthropod-borne viral disease in subtropical and tropical regions of the world. The dengue virus (DENV) belongs to the *Flaviviridae* family and consists of four antigenically distinct serotypes (DENV1-4).[1] The infection can result in a broad spectrum of effects, including acute febrile illness, the dengue fever (DF), which may progress to severe forms such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), with changes in hemostasis and vascular permeability. Benjamin Rush in 1789 called it Break Bone Fever. In 1906, Aedes mosquitoes transmitting the dengue fever was confirmed and in 1907, Dengue was the second disease after "yellow fever" that was shown to be caused by virus. Dengue hemorrhagic fever is first reported in Philippines in 1953, and in 1981 in South America.[2]

Besides the occurrence of hemorrhage and edema in the liver of dengue fatal cases, histopathological analysis also reported damages caused by metabolic alterations and/or inflammatory reactions, such as the presence of steatosis, areas with infiltrated cells and necrosis and hyperplasia and destruction of Kupffer cells.[3]Involvement of younger age group with increasing frequency in epidemics are indicators of higher incidence of infection. If untreated, mortality from complications of DF is as high as 20%, whereas early case detection and management, decreases mortality to <1%, especially in children. Dengue specific IgM and IgG ELISA are widely used for diagnosis and exposure to dengue.[4] The present study was conducted to assess the effectiveness of antibodies in diagnosing cases of dengue fever.

MATERIALS & METHODS

The present study comprised of 142 patients of dengue fever. The study protocol was approved from institutional ethical committee. All were informed regarding the study and written consent was obtained.

In all patients, a through clinical examination was performed. Duration of fever was recorded. Platelet count was calculated. For detection of NS1 antigen, IgG and IgM antibody rapid immune chromatography

method was used. 100 µL of patient's serum was added to NS1 and 10 µL to IgM/ IgG device for 20 minutes. Results thus obtained were tabulated and subjected to statistical analysis. P value<0.05 was considered significant.

RESULTS

Table I shows that out of 142 patients, males were 82 and females were 60.

Table II shows common clinical features were headache in 76, vomiting in 89, abdominal pain in 94, myalgia in 52, hepatomegaly in 48 and bleeding in 36 cases. The difference was significant (P<0.05).

Table III, graph I shows that among serum samples, 90% found positive for NS1, IgG (4%), IgM (2%). 1.3% for both NS1 and IgG, 1.2% for NS1 and IgM and 0.50% for NS1, IgM and IgG. The difference was significant (P<0.05).

Table I: Distribution of patients

Total- 142		
Gender	**Male**	**Female**
Number	82	60

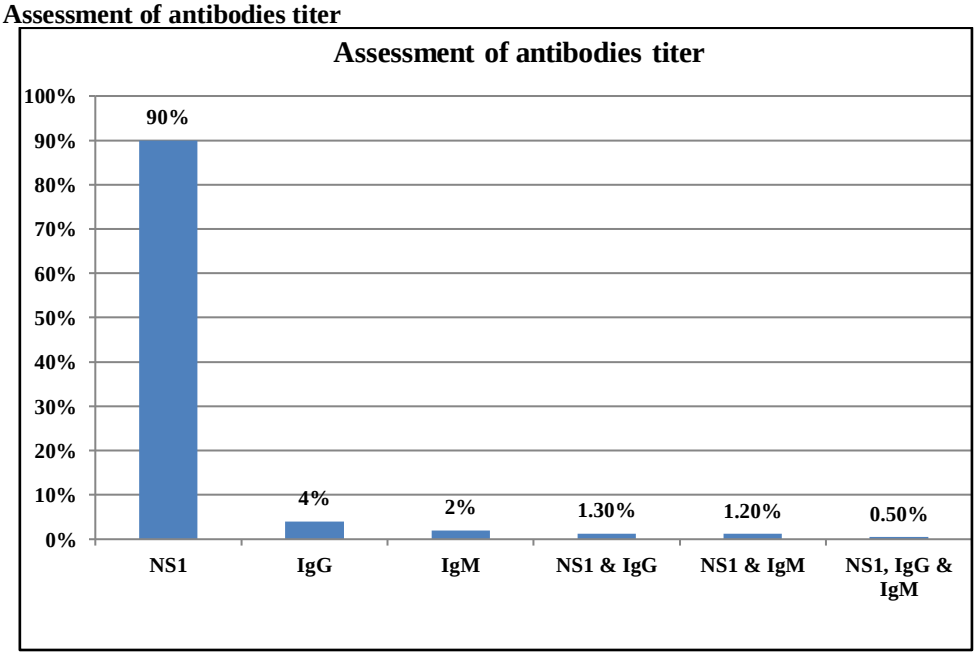
Table II: Clinical manifestations of patients

Clinical features	Number	P value
Headache	76	0.05
Vomiting	89	
Abdominal pain	94	
Myalgia	52	
Hepatomegaly	48	
Bleeding	36	

Table III: Assessment of antibodies titer

Antibodies	Percentage	P value
NS 1	90%	0.01
IgG	4%	
IgM	2%	
NS 1 & Ig G	1.3%	
NS 1 & Ig M	1.2%	
NS 1 & Ig G & Ig M	0.50%	

Graph I: Assessment of antibodies titer



DISCUSSION

Dengue is believed to infect 50 to 100 million people worldwide in a year. The mortality is 1-5% without treatment and less than 1% with treatment.[5]Dengue is a major challenge to public health, especially in South-East Asia. It has a wide geographical

distribution and can present with a diverse clinical spectrum. 2.5 billion people worldwide live in areas where there is a significant risk of infection by the dengue virus.[6] Infection with the virus can cause a spectrum of illnesses including relatively mild disease with fever, known as classic dengue fever (DF) and more severe forms such as dengue hemorrhagic fever (DHF), dengue shock syndrome (DSS) and less frequently acute hepatitis, disseminated intravascular coagulation, encephalopathy, myocarditis, acute renal failure and hemolytic uremic syndrome both in adults and children.[7] The present study was conducted to assess the effectiveness of antibodies in diagnosing cases of dengue fever.

In present study, out of 142 patients, males were 82 and females were 60. Idirisinghe et al[8] conducted a study in which tissue from seventeen autopsies following DHF were processed routinely and stained with haematoxylin and eosin. Histopathological changes in the liver, lungs, heart, brain, spleen, gastrointestinal tract and kidneys were studied. Fifteen of 17 cases showed a liver pathology which included submassive necrosis (1/15), bridging necrosis (2/15), midzonal (3/15) and centrilobular necrosis (4/15), focal necrosis (2/15), apoptosis (1/15) and macrovesicular steatosis (7/15). Pulmonary hemorrhage was present in 13 cases and pulmonary oedema in 6 cases. Glomerular congestion and hemorrhage were seen in 6 and medullary congestion with hemorrhage was present in 9 cases. Gastrointestinal mucosal hemorrhages were seen in 8. One patient showed myocarditis and one showed myocardial hemorrhages. Cerebral oedema was present in 8 cases In 13 out of 17 cases the spleen showed red pulp congestion and hemorrhage.

We found that common clinical features were headache in 76, vomiting in 89, abdominal pain in 94, myalgia in 52, hepatomegaly in 48 and bleeding in 36 cases.Liver is the most commonly affected organ in DHF and liver injury in DHF has been described. Lee et al[9] studied 6 autopsies of DHF in India and all cases showed derangement in liver function tests indicating 100% liver involvement in their study. Raised levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) have been observed in 98% and 37% of dengue patients, respectively.

We found that among serum samples, 90% found positive for NS1, IgG (4%), IgM (2%). 1.3% for both NS1 and IgG, 1.2% for NS1 and IgM and 0.50% for NS1, IgM and IgG.Panchareon C et al[10] conducted study on 112 subjects and found that NS1 found in 77% of subjects as early as on 2nd day of fever. Complications of dengue fever are not uncommon. Withayathawornwore[10]found thrombocytopenia in 45% of NS1 positive patients, 52% of IgG positive patients, 65% of IgM positive patients, 31% of both NS1 and IgM positive patients, 100% of NS1, IgG and IgM positive patients. Wilder[11] in his study found that thrombocytopenia most commonly seen among IgG positive patients. Pal S et al evaluated these tests using a well-characterized panel of clinical samples to determine their effectiveness for early diagnosis. Retrospective samples from South America were used to evaluate the following tests: (i) “Dengue NS1 Ag STRIP” and (ii) “Platelia Dengue NS1 Ag ELISA” (Bio-Rad, France), (iii) “Dengue NS1 Detect Rapid Test (1st Generation)” and (iv) “DENV Detect NS1 ELISA” (InBios International, United States), (v) “Panbio Dengue Early Rapid (1st generation)” (vi) “Panbio Dengue Early ELISA (2nd generation)” and (vii) “SD Bioline Dengue NS1 Ag Rapid Test” (Alere, United States). Overall, the sensitivity of the RDTs ranged from 71.9%–79.1% while the sensitivity of the ELISAs varied between 85.6–95.9%, using virus isolation as the reference method. Most tests had lower sensitivity for DENV-4 relative to the other three serotypes, were less sensitive in detecting secondary infections, and appeared to be most sensitive on Day 3–4 post symptom onset. The specificity of all evaluated tests ranged from 95%–100%. ELISAs had greater overall sensitivity than RDTs.[12] Wichmann O et al evaluated the diagnostic value of positive dengue antibody-titres performed by a standard ELISA (PanBio IgM- and IgG-ELISA) in single serum samples (regarded as "probable infection"). A total of 1,035 febrile travellers returning from dengue-endemic countries with negative dengue-serology and RT-PCR served as controls to compare clinical and haematological features. Overall, only 64 (positive predictive value = 50%) of the probable cases were confirmed by additional analysis and 54 (42.5%) were confirmed to be "false-positive". Rash was the only clinical feature significantly associated with confirmed dengue fever. The combination of thrombocytopenia and leucopenia was present in 40.4% of confirmed and in 6.1% of false-positive cases. Thus, the positive predictive value for the combination of positive PanBio-ELISA plus the two haematological features was 90.5%. The examination of paired serum samples is considered the most reliable serodiagnostic procedure for dengue.[13]

Jayathilaka D et al investigated the immunoglobulin responses of patients with dengue fever (DF) and dengue hemorrhagic fever (DHF) to NS1. Antibody responses to recombinant-NS1 are assessed in serum samples throughout illness of patients with acute secondary DENV1 and DENV2 infection by ELISA. NS1 antibody titres are significantly higher in patients with DHF compared to those with DF for both serotypes, during the critical phase of illness. Furthermore, during both acute secondary DENV1 and DENV2 infection, the antibody repertoire of DF and DHF patients is directed towards distinct regions of the NS1 protein. In addition, healthy individuals, with past non-severe dengue infection have a similar antibody repertoire as those with mild acute infection (DF). Therefore, antibodies that target specific NS1 epitopes could predict disease severity and be of

potential benefit in aiding vaccine and treatment design.[14]

CONCLUSION

Dengue fever is common nowadays. It was found that NS1, IgG and IgM are diagnostic marker for dengue fever.

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