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Microbiology

CORRELATION OF THROMBOCYTOPENIA AND SEROLOGICAL MARKERS IN DENGUE INFECTION.

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ABSTRACT

Introduction: Dengue is the most common and widespread arboviral infection. It is a flaviviral infection found in large areas of tropical and subtropical regions of the world. Early and specific diagnosis of dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS), followed by supportive therapy, reduces morbidity and mortality. NS1 Detection is reported to be sensitive as well as specific test.Apart from the dengue-specific parameters platelet count is one of the important predictive markers to help in early diagnosis of Dengue infection. In primary care setup, platelet count is only additional test available that can support the diagnosis of DHF or DSS. In present study we tried to correlate the platelet count and immunochromatography (ICT) based dengue serology test.

Aims: To assess platelet count in serologically positive dengue cases.

Methods: A total of 750 serum samples collected from clinically suspected dengue fever patients were collected. Samples tested for NS1, immunoglobulin M (IgM),G(IgG) by ICT-based test. The platelet count was noted in all the positive dengue cases.

Results: A total 50 cases showed positivity for either one and more of the three markers (NS1, IgM, IgG). A Platelet count <1,00,000/ml was observed in 33(66%) cases.The association of thrombocytopenia in NS1+IgM positive cases was statistically significant (Z=2.057, P<0.0394).

Conclusion: Rapid immunochromatography (Both NS1, & antibodies detection) test is an excellent tool in the diagnosis of dengue increases the detection rate significantly. In our study , thrombocytopenia was seen in statistically significant number of patients having NS1 and IgM positivity simultaneously.

KEYWORDS : Dengue, NS1, IgM, IgG , Thrombocytopenia

INTRODUCTION:

Dengue is the most common and widespread arboviral infection in the world today, also called classic dengue or break bone fever. It is a flaviviral infection found in large areas of tropical and subtropical regions with significant morbidity and mortality.[1] Dengue virus is an enveloped, single-stranded, positive sense RNA virus belonging to the genus *Flavivirus* in family *Flaviviridae* . There are four serotypes of dengue virus (DV), namely DEN-1,DEN-2, DEN-3, DEN-4.

In humans, one serotype produces lifelong immunity against reinfection but only temporary and partial immunity against the other serotypes.[2]All four serotypes can cause the full spectrum of disease ranging from a mild self-limiting disease, the dengue fever (DF), to life-threatening dengue hemorrhagic fever and dengue shock syndrome.[3] World Health Organization (WHO) has taken several preventive measures to control the spread of dengue virus infection. However, still new outbreaks are reported in several parts of the world during post monsoon season. Newer diagnostic techniques, public awareness programs, better education, and proper monitoring of vector control are required to prevent such outbreaks.

The transmission of dengue is dependent on various macro and micro level factors such as temperature, humidity, rainfall, the population density, movement, immunity and virus load, urbanization, environmental factors and socio-demographic and economic factors.[4] These influence the spread of the disease through increased *Aedes aegypti* mosquito population, transmission of the vector, spreading of the disease and practices of protection mechanism, Vector dynamics.[5] It is important that, early case detection and prompt diagnosis followed by supportive management reduces morbidity and mortality.[6] Currently the three basic methods used by most laboratories for the diagnosis of dengue virus infection are viral isolation, detection of the viral genomic sequence by a nucleic acid amplification technology assay (Reverse transcription polymerase chain reaction (RT-PCR)), and detection of Dengue specific IgM antibodies and antigen by the IgM –Capture enzyme linked immunosorbent assay (MAC-ELISA) and /or the rapid dengue immunochromatographic test (ICT).[7]

Detection of NS1 has been a promising test to diagnose dengue in its early febrile stage due to its long half-life in blood. The NS1 protein was found to be highly conserved in all dengue serotypes, circulating in high levels during the first few days of illness. It correlates with the development of DHF. There is no cross-reaction of the dengue NS1 protein with those of other related *flaviviruses*.[8]Apart from the dengue-specific antigen and antibodies, platelet count is one of the important predictive markers to help in early diagnosis of Dengue infection.[9]In primary care setup, platelet count is only additional test available that can support the diagnosis of DHF or DSS. A rough estimation of platelet counts by microscopy in resource-limited settings of primary care is helpful in diagnosis and monitoring the treatment of infection. In the present study, we have correlated the platelet counts, and ICT-based dengue serology tests which will help the clinicians to diagnose and monitor the treatment of DENV infections.

MATERIALAND METHODS:

This was prospective observational study conducted in the Department of Microbiology**,** IIMSR Warudi , Badnapur - a tertiary care hospital from July 2020 to December 2020 after receiving permission from the institutional ethical committee. A total of 750 serum samples collected from clinically suspected cases of dengue –like illness attending the outpatients departments and admitted in inpatient departments and sent for serological diagnosis of Dengue infection were included in this study.

Samples were tested by a rapid qualitative immunochromatographic assay (J.Mitra andCo Pvt Ltd, Dengue Day 1 test) for differential detection of dengue specific IgM and IgG antibodies and NS1 antigen. Platelet counts of all the positive cases for any of the dengue parameter were recorded by cell counter method.(Sysmax haematology analyser Xn350)

RESULTS:

Total 750 serum samples from suspected dengue cases were collected and subjected to immunochromatography test (ICT). A total 50 cases showed positivity for either one and more of the three markers (NS1,IgM, IgG) as shown in **(table 1).** Majority of 36 (72%) Cases were positive for NS1 followed by IgM 5(10%) and IgG 4 (6%) respectively. More than one marker was detected in the remaining 7

(14%). All the suspected dengue positive cases were screened for platelet count. Among them in 33 (66%) cases platelet counts were less than 1,00,000/cmm. **(Table2)** of which maximum found in NS1.

DISCUSSION:

DF is an acute febrile arthropod-borne illness mainly affecting the tropical and subtropical countries.[10]A rapid and sensitive laboratory methods are required for early detection of the disease to reduce the morbidity and mortality.

Detection of Dengue-specific antibodies had been the mainstay of diagnosis for a long time though these antibodies usually appear near the end of a week of primary infection. In majority of the secondary infections, both IgM and IgG antibodies do not appear before the 3rd day, thus creating a window period in both the types of infection.To overcome this, a new parameter available for diagnosis is NS1 antigen which acts as a highly specific viral marker for the diagnosis of DIs. NS1antigen is detectable in the serum from day 1 of fever in both primary and secondary infections.[9]

Thrombocytopenia is not an early indicator of severe disease in dengue infection but it helps in predicting the progression of disease. Platelet count are decreased in several other conditions such as some viral infections other than dengue, drug induced thrombocytopenia, collagen vascular diseases, and idiopathic thrombocytopenia.[11] Therfore, association of platelets counts in cases of fever with dengue is an important marker to determine the severity of infections.

A 'gold standard' tests for diagnosis of dengue infection (DI) are not within the reach of many tertiary care laboratories. The mainstay of diagnosis of DI is detection of dengue - specific antibodies IgG/IgM. However, antibody detection is an indirect method of diagnosis and therefore it prone to false positive as well as false negative results. NS1 detection is reported to be sensitive and highly specific test.[12]

In this study, of 50 cases, 35(70%) were positive for only NS1 antigen. As Ns1 test is highly specific it can be stated that we would have missed the diagnosis in 70% cases if we had not included NS1 in the test panel. Kanthikar et al [13], Kulkarni et al[14], Datta et al [8], Shrivastava et al[7] have shown that NS1 was positive in 45 (33.33%) ,95 of 320(30%),140 of 600 (23.3%), and 15 of 91 (16%) cases, respectively. In this study, NS1 detection rate was 70%, which is much higher than the findings reported by other authors. This may be attributed to the collection of large number of samples at the time of peak season of the infection. However, Jyoti and Metri [15] and Mehta et al [9] have detected an almost similar rate of NS1 only positivity (62.9% and 64.5%, respectively) in their studies.

In our study , NS1 alone or in combination with either IgM or IgG was positive in 41 cases (82% cases). The NS1 antigen is a highly specific marker of dengue infection, as there is no cross reaction of dengue NS1 protein, with those of other related flaviviruses.[7,8] The DENV IgM as well as IgG antibodies show some cross-reactivity with other members of the *flaviviridae* family. Utility of antibody in the diagnosis of infections relies mainly on rising titres, especially in the endemic areas. Among two antibodies, IgG is a less reliable marker in the diagnosis of DI as it can be detected in clinical as well as subclinical DIs for several years.[16] Furthermore, it can be said that the level of IgG antibodies could be higher in endemic areas because of bites from infected mosquitoes.[17]

However, dengue-specific IgM is a very good indicator of recent infection. It may also be detectable in secondary Dengue Infection.

In our study, we observed IgM and IgG is 5 (10%), 3(6%) respectively, We determined the association of positive dengue parameters with thrombocytopenia. The cases positive for NS1 antigen alone or with both antibodies showed no association with thrombocytopenia. However,NS1+IgM-positive cases showed a statistically significant association with thrombocytopenia (Z=2.057, P<0.0394).

A study by Kulkarni et al.[14]detected a consistent association of NS1 with thrombocytopenia when compared to antibodies**.** In contrary, Mehta et al.[9] have found a higher association of IgM antibody with thrombocytopenia, and Golia et al.[18] have detected a higher association of IgG antibody with thrombocytopenia. The role of antibody in the pathogenesis of DF is well-known.

Table: 1 Comparison of dengue parameters

Parameters	n(%)
NS1 only	35 (70%)
IgM only	5(10%)
IgG only	3(6%)
NS1+ IgM	4(8%)
NS1+ IgG	2(4%)
IgM+IgG	1(2%)
NS1+IgM+IgG	0
Total	50 (100%)

Table:2 Comparison of platelet count and dengue parameters

Parameters	n (%)	Platelet count (<100,000)(%)
NS1 only	35 (70%)	24 (67%)
IgM only	5(10%)	3(60%)
IgG only	3(6%)	2(66.7%)
NS1+ IgM	4(8%)	3(75%)
NS1+ IgG	2(4%)	1(50%)
IgM+IgG	1(2%)	0
NS1+IgM+IgG	0	0
Total	50 (100%)	33(66%)

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