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DENGUE: SEROLOGICAL AND HEMATOLOGICAL CORRELATION IN A TERTIARY CARE HOSPITAL IN HYDERABAD

Medicine

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ABSTRACT

Aim: Dengue fever is one of the emerging epidemic occurring in tropical and subtropical regions. As there is no specific antiviral therapy,early diagnosis helps to reduce the morbidity and mortality. Our study is to analyse the utility of hematological parameters along with serological patterns in dengue.

Materials and methods: A retrospective study was done for a period of one month in November2019 in the Dept of Pathology, Princess Esra Hospital, Deccan college of Medical Sciences,Hyderabad. Suspected dengue cases were evaluated for serology and hematological investigations.

Results: Out of 762 suspected cases,298 cases were confirmed with serology of which 223(75%)were positive for NS1 with/without antibodies. Raised NS1 was associated with hematocrit, normal WBC count, thrombocytopenia & lymphocytosis whereas neutrophilia was linked to antibodies.

Conclusion: In resource limited setups,haematological parameters and serology are helpful as early markers of dengue and aid in early diagnosis and management.

KEYWORDS

Dengue,NS1 antigen, antibody, platelet count

INTRODUCTION:

Dengue is an arthropod borne disease caused by dengue virus and is a major public health problem in most of the countries[5] DENV belongs to the family Flaviviridae, and it is transmitted to humans by the Aedes aegypti mosquitoes.[10] Its caused by four antigenically (related but distinct serotypes DENV-1, DENV-2, DENV-3, and DENV-4) [28]. Majority of the cases of DENV infections may remain asymptomatic, but few may present with a wide range of clinical symptoms, ranging from mild dengue fever to a severe and sometimes fatal form of the disease known as DHF and DSS[18] .It affects up to 100 million people annually, with 5, 00,000 cases of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) and around 30,000 deaths, mostly among children[13].

The "gold standard" tests for dengue are virus isolation, detection of viral genome sequence by reverse transcription polymerase chain reaction (PCR), and detection of DEN specific immunoglobulin M (IgM) antibodies and NS1 antigen by enzyme linked immunosorbent assay (ELISA) and/ or rapid dengue immunochromatographic test (ICT)[30]. However in resource limited settings as in primary health care, diagnosis usually depends on simple to perform and interpret diagnostic test to prevent the morbidity and mortality caused due to DHF and DSS[15]. NS1 is expressed on the surface of infected cells without forming part of the virion. Serum levels of NS1 positively correlate with viral titers and have been a useful tool in diagnosis[4] and is reported to be sensitive as well as highly specific[31].

Detection of dengue specific IgM/ IgG has been the mainstay of diagnosis but their detection is an indirect method of diagnosis and therefore is prone to false positive as well as false negative results.

Apart from the dengue specific antigen and antibodies, platelet count is one of the important predictive markers to help in early diagnosis and monitoring the treatment of infection[19]. Haematocrit rises due to hemoconcentration attributed to plasma leakage as a result of increased capillary permeability also aids in prognosis and management in dengue. Leucopenia is a major finding in dengue & caused by bone marrow suppression by virus.

MATERIALAND METHODS:

This was a retrospective study done for a period of one month in November 2019, in the Department of Pathology in collaboration with Department of Microbiology at Princess Esra hospital, Deccan college of Medical Sciences, Hyderabad. In the present study, we have tried to correlate hematological and serological parameters in suspected dengue cases.

A total of 762 serum samples were collected from clinically suspected cases of dengue like illness and screened for NS 1 antigen, anti dengue IgM and IgG antibodies by ELISA. All the seropositive cases were screened for various hematological parameters using SYSMEX XN 550 automated cell counter and all the abnormal parameters were cross checked manually.

RESULTS:

Of the 762 serum samples tested, a total of 298 samples were found to be seropositive for dengue parameters. The number of males effected were 174 (58 %) and females were 124 (42 %) with male: female ratio of 1.4 :1.The most effected age group was 11-30 years which contributed to 59 % of the cases with average age being 29 years. The youngest patient was of 5 months and the eldest one to be effected was of 74 years of age. (Table 1)

Table 1: Age wise distribution of seropositive cases

Age group	No. of cases	%
0-10	76	26
11-20	98	33
21-30	77	26
31-40	28	09
41-50	08	03
51-60	07	02
61-70	03	01
71-80	01	0
Total	298	100%

Of the 298 serum samples, 204 (69 %) patients were positive for NS1 only, 34 (11 %) positive for IgM only, while 6 (2 %) patients had only IgG. More than one marker was detected in the remaining 54 (33 %) samples. In our study, primary infection (positive for NS1 Ag, IgM, NS1 + IgM) was seen in 245 (82 %) cases and secondary infection (positive for IgG, NS1 + IgG, IgM + IgG, NS1+ IgM+ IgG) was seen in 53 cases (18%).

Raised haematocrit values were observed in 161 cases (54 %) of patients while 137 (46 %) had haematocrit values within normal range There was a higher proportion in NS1 with/without antibody with 109 cases(68%) as against the lower proportion of 52 cases(32%) with

only antibody parameter (Table 3)

Table 2: Prevalence of dengue parameters

Parameter	No. of cases	%
NS 1	204	69
Ig M	34	11
Ig G	06	02
IgM / IgG	35	12
NS 1/IgM	07	02
NS 1/IgG	01	0
NS 1/ IgM/ IgG	11	04
Total	298	100%

Table 3: Haematocrit and serological correlation:

Parameter	No. of cases	%	Hct and serology association
NS 1	48	30	68 %
NS I with Antibodies	61	38	
Antibodies	52	32	32%
Total	161	100	

In our study, out of 298 positive cases, leucopenia was observed in 97 (33 %) of patients while 29 (9 %) had leucocytosis. Total leucocyte count within normal range was seen in 172 cases(58 %) (Table 4).Out of 97 cases of leucopenia, 63 cases(65 %) are observed in association with NS1 antigen pattern with and without antibodies whereas 34 cases(35 %) were noted in antibody only pattern(Table 5).In contrast normal counts were linked to NS1 with and without antibody which contributed to approx 152 cases (88%) and 20 cases (12%) in the antibody only pattern. Leucocytosis was a later event mostly associated with antibody contributing to 72% of cases when compared to NS1 with or without antibodies which showed 28 % . (Table 6)

Table 4: Total leucocyte count:

Total WBC count	No. of cases	%
Normal count	172	58
Leucopenia	97	33
Leucocytosis	29	9
Total cases	298	100

Table 5: Leucopenia in association with serology

Pattern	Number	%	Ns1 Ab association
Ns1 antigen	52	54	65%
Ns1 with antibodies	11	11	
Antibodies only	34	35	35%
Total	97	100	100

Table 6: Normal / increased WBC in association with serology:

Parameter	Normal WBC		Increased WBC	
	Number	%	Number	%
NS 1	148	86	4	14
NS 1 with Antibodies	4	2	4	14
Antibodies only	20	12	21	72
Total	172	100	29	100

Differential count was changed in 213 of 298 cases (72 %) and was normal in 85 cases (28%), Out of 213 cases, we observed lymphocytosis in 196 (66%) and neutrophilia in 17 (6%) of cases. A significant number of 67% were associated with NS1 antigen pattern in cases with lymphocytosis. Neutrophilia was more predominant in Antibody only pattern contributing to 59 % of cases (Table 7).

Table 7: Differential count in dengue positive cases

Serology patterns	Neutrophilia		Lymphocytosis*	
	Number	Percent	Number	Percent
NS1 antigen	5	29	133	67
NS1 with antibody	2	12	13	7
Antibody only	10	59	50	26
TOTAL	17	100	196	100

Of 298 positive cases, thrombocytopenia of < 1 lac/cumm was found in 213 (72 %) cases while 85 cases(28 %) were found to have platelet count of more than 1 lac/cumm(Table 8).

We tried to find the association of dengue parameter positivity with thrombocytopenia. The comparison of platelet counts with different dengue specific parameters is shown in (Table 9). In 223 cases that were positive for NS1, thrombocytopenia was observed in 165 cases (56 %) cases. In contrast, when only antibodies were considered for the diagnosis of dengue infection, thrombocytopenia was noted in 48 cases (16 %) cases only.

Table 8: Platelet count of dengue positive cases.

Platelet count	No. of cases	%
Platelet count < 1lac/cumm	213	72
Platelet count>1lakh/cumm	85	28
Total	298	100

Parameter	No. of cases	Plt < 1 lac	NS 1 -Ab association
NS 1	204	153	56 %
NS 1 with antibodies	19	12	
Antibodies only	75	48	16 %
TOTAL	298	213	72 %

DISCUSSION:

Of the 762 serum samples tested, a total of 298 samples were found to be seropositive for dengue parameters. The number of males effected were 174 (58%) and females were 124 (42 %) with male : female ratio of 1.4 :1.Our study analysis was found to be similar to the one done by Anagha A et al[3]. , Ingale sv et al [11] and by Mohan et al[20] which was found to be 1.2:1

Of all the seropositive cases, the most effected age group was of 11-30 years which contributed to 59 % of the cases with average age being 29 years. This study was compatible by that done by Krunal et al[21]. The youngest patient was of 5 months and the eldest one to be effected was of 74 years of age.

During primary infection, IgM appears after 5–6 days and IgG after 7–10 days. During a secondary infection, high levels of IgG are detectable even during the acute phase whereas IgM are low or absent [7,9] . Viral nonstructural 1 (NS1) antigen is abundant in the serum of patients in the early stages of dengue infection, lasting from 1 to 9 days; therefore, NS1 antigen especially when used together with IgM is sufficiently informative in an acute condition [6]

In our study ,out of 298 seropositive cases, total number of cases with positivity for NS1 alone or in combination with antibodies were found in 223 cases (75%), out of which only NS1 positive cases were found to be 204 cases (69%) and NS1 with IgM, IgG or both contributed to 19 cases (6%). Only Antibodies were positive in 75 cases(25 %). The study conducted by Jyoti et al [13] had similar results with 74% cases of NS1 with or without antibodies with only NS 1 contributing to 63 % of cases. Other studies done by krunal et al[21], qursheed et al[24],sneha et al[25] and Ingale et al[11] had similar findings with NS1 contributing to more than 60 % of the cases .

In our study, primary infection (positive for NS1 Ag, IgM, NS1 + IgM) was seen in 245 (82%) cases and secondary infection (positive for IgG, NS1 + IgG, IgM + IgG, NS1+ IgM+ IgG) was seen in 53 cases (18%).Studies done by Sneha et al, Jyoti et al and Ingale et al had similar findings.[25,13,11]

Haematocrit rises due to hemoconcentration attributed to plasma leakage as a result of increased capillary permeability occurring in the critical period. It aids in prognostication and management in dengue[17,23]. Analysis of our study showed 161cases (54 %) with a rise in haematocrit, showing a predominant association with NS1 with/without antibody (68%) as against the lower proportion (32%) with Antibody alone .Our studies had similar findings with that done by Anagha A et al[3] and Ingale SV et al[11] where they found the HCt values to be raised in 55 % and 49 % respectively. However the studies done by Surendra Nath et al[26] and Tahir et al [27] had only 23 % and > 20% of patients with high haematocrit levels.

Total leucocyte count - Leucopenia is a major finding in dengue & caused by bone marrow suppression by virus[14,22] and aids in diagnosis, differentiation & prognostication of dengue [1,2,27]. In our study, we had 97 cases of leucopenia with 63 cases(65%) of these observed in NS1 antigen pattern with and without antibodies whereas 34 cases(35%) were noted in antibody only pattern. There was a strong association

with NS1 positivity which was in concordance with other studies[3,24]. In contrast normal counts were noted in 152 cases (88%) in NS1 with/without antibody pattern and antibody only pattern contributed to approx 20 cases (12%). Leucocytosis was a later event mostly associated with antibody contributing to 21(72%) of cases when compared to NS1 with/without antibodies which showed 28% with 8 cases.

The results of our study suggests that leucopenia had a strong NS1 association and is an early marker of dengue infection and leucocytosis was seen predominantly with Antibody only parameter which correlated with the study done by Anagha A et al[3].

Differential count - Bone marrow suppression causes a decrease in polymorphs with increase in lymphocytes especially atypical lymphocytes due to stimulation by nonspecific or specific viral antigens[2,16].The differential count (especially lymphocytosis) helps in differential diagnosis & prognostication in dengue[14,22].

We observed lymphocytosis in 196(92%) and neutrophilia in 17(6%)of cases amongst 213cases with abnormal differential counts. Lymphocytosis was seen in 146(74%) in association with NS1 with/without antibodies.. Neutrophilia was seen predominantly linked with antibody only pattern contributing to 59% of cases.

Our study supports the observation of few studies that early in illness there is decrease in neutrophils with lymphocytosis[16]. Study done by Anagha A et al[3] had similar findings.

Thrombocytopenia in dengue is caused by bone marrow suppression & immune mediated clearance of platelets[29]. It aids in diagnosis of dengue & helps in its differential diagnosis[14]. It is useful as a diagnostic, predictive & recovery parameters of DHF[12].

We had a total of 213(72%) cases of thrombocytopenia with platelet count less than 1 lac/cumm. The results in severe thrombocytopenia category showed higher proportion of cases with NS1 antigen pattern with and without antibodies contributing to 56 % of cases followed by antibody only parameters with 16%. This was in concordance with few studies which claimed increased association with NS1 antigen[15,11] .Some studies showed increased association of thrombocytopenia with antibody patterns[3,19]Others claimed more association of NS1 with antibody[8]. A few studies have found no correlation between the two[13]. However, on comparison between studies, it was found inaccurate due to varying thresholds of platelet count, varying methods of correlation, inclusion of normal & high platelet counts in the studies & number of cases included in the study.

The results in our study was in agreement with the observation made in few studies that platelet counts start decreasing by 3rd/4th day to 7th day[3,12].

CONCLUSION:

In India, frequent dengue epidemics strain the limited resources of the public health system. The highly sensitive techniques such as viral culture and PCR are not easily available for the diagnosis of Dengue infection. The resource poor health-care system has to depend on simple to perform and easy to interpret laboratory tests for diagnosis. This study explores the utility of simple, easy, rapid & cost effective tests like serology & hematology, also as a supplement & substitute to one other in diagnosis & prognosis of the dengue spectrum.

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