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Research Article

A STUDY ON EVALUATION OF CORRELATION BETWEEN DENGUE SEROLOGICAL MARKERS AND PLATELET COUNT FOR EARLY DETECTION

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ABSTRACT

Dengue is an important public health problem worldwide. Dengue virus infection can produce self-limiting illness to fatal life threatening complications like dengue hemorrhagic fever [DHF] and dengue shock syndrome [DSS]. Dengue specific NS1 antigen, IgM and IgG antibody detection can be used for early diagnosis which is essential for effective management of cases to reduce the mortality and morbidity. The only non-dengue parameter which helps in predicting complications is platelet count. Therefore we tried to evaluate the association of platelet count against NS1 and lgG/lgM in dengue infections. The study includes detection of dengue NS1antigen by ELISA in patients having 1 to 5 days fever, detection of dengue NS1 antigen and IgM antibody by ELISA in patients having fever of five to nine days and only detection of IgM antibody by ElISA in patients having fever of more than 9 days. Platelet count of the positive patients for dengue NS1 antigen and IgM antibody by ELISA were seen. The present study was done in department of Microbiology Osmania General Hospital, Hyderabad during year March 2015 to October 2015. 426 serum samples from patients with a suspecting of dengue infection were collected. These patients were divided into three groups according to days of fever Group I - Patients having 1 to 5 days fever were tested for NS1 antigen ELISA. Out of 132 tested 35(26.5%) patients were positive for dengue NS1 antigen. Group II: Total patients having fever more than 5 days to 9 days were 106. Out of 106 samples, 44 (41.5%) were positive either for NS1 ag or IgM ab or both. A total of 16 (15%) samples were positive for NS1 Ag and 33 (35%) were positive for IgM ab including t significantly increased the detection rate to 41.5% .those that were positive by both. Both the assays, performed together on a single serum sample. GROUP III: 188 patients were having fever of more than 9 days. These were tested for only Dengue IgM ELISA. 68(36.1%) patients were showing positive for dengue IgM by ELISA. Platelet counts of patients positive for dengue NS1 antigen and IgM were noted. Platelet count < 1 lac /cumm is considered as Thrombocytopaenia, which was seen in 87% of seropositive cases. Mild TCP observed in 29.6 % of seropositives**.** TCP was seen in 30.8% of seronegative cases. More no. of Seronegatives (24.4 %) was observed between 61,000-80,000/cumm Platelet count. TCP with seropositivity (87.7%) and seronegativity (30.8%). Mild TCP noticed in highest no.of patients 29.1, followed by Moderate in 16.4% and severe in only 4.6% of cases. Platelet count <1 lac/cumm was associated significantly when both serological markers were detected (95%) than with individual markers NS1 (91%) and IgM (80.7%). In the present study, 91.4% TCP was noticed in NS1 seropositive. TCP was noticed in 95.4% when simultaneous detection of NS1& Ig M done. Decrease in Platelet count seen in 80.8 % of Ig M seropositives. The study concluded that combination of dengue NS1 antigen and IgM ELISA on single sample can improve the diagnosis of dengue without the requirement of paired sera. Correlations of positive samples with platelet counts were noted. Apart from dengue specific marker parameter platelet count is the only accessory laboratory test available that can support the diagnosis for dengue. Association of thrombocytopenia in dengue parameter positive cases was highly significant when compared to thrombocytopenia in dengue parameter negative cases.

INTRODUCTION

Dengue has become a major global public health problem in the developing countries. The estimated risk of acquiring Dengue virus infection (DV) is approximately 2.5 billion people living mainly in urban areas [1]. DV causes various clinical symptoms ranging from asymptomatic or undifferentiated fever, known as dengue fever (DF), dengue hemorrhagic fever (DHF), dengue shock syndrome (DSS), leading to death, especially among children [2]. Dengue is an acute febrile illness, which is endemic in India. Dengue virus infection is transmitted by Aedes aegypti mosquito and the virus belongs to the family *Flaviviridae,* Four serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) can be identified on the basis of neutalisation tests. Dengue virus infection affects 100 million population annually. Due to rapid urbanization, lifestyle changes and deficient water

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management, the risk of dengue infection in India has increased in recent years [3].Dengue virus infection in human beings may result in varied clinical illness ranging from subclinical infection to non-specific febrile illness, classic dengue fever, dengue haemorrhagic fever [DHF- grades I and II], and dengue shock syndrome (DSS - grades III and IV) [4]. The combined case fatality rate is around 5% for dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [5]. Hence early, rapid and accurate diagnosis is essential for appropriate clinical management to reduce the mortality rate.

At present scenario effective antiviral treatment and vaccine is not available in India. Rigorous intravenous fluid management and high index of suspicion of complications is only treatment modality. Diagnosis by RT-PCR is not available in most of the hospitals, as it is expensive.5-7 In most of the tertiary care laboratories and the periphery the "gold standard" for identification of Dengue Infection are not available. Thus, in the periphery, dengue diagnosis is mainly by detection of IgG/IgM antibodie and NS1 antigen sare known to give false positive and false negative results.

Virus isolation in cell culture, identification of viral genomic sequence by nucleic acid amplification techniques like RT-PCR, NASBA and detection of dengue specific IgM, IgG antibodies are widely used by most of the laboratories for diagnosis of dengue infection [9]. Virus isolation and molecular techniques cannot be used as routine diagnostic tests because they are laborious, time consuming and require specialized laboratory facilities [6]. Serological diagnosis by detection of antibodies is widely used, but antibodies appear only after 4 to 6 days of illness [11]. Secretory protein NS1 antigen is seen in high concentrations during acute phase of illness (1 to 5 days) [12]. Combination of NS1 antigen detection along with antibody detection increases the diagnostic rates [7]. Immunochromatographic detection of these serological markers yield rapid results but have low sensitivity as compared to ELISA [14]. During first 3 days of illness platelet count is normal. Thrombocytopenia begins during febrile phase and platelet count is progressively reduced during hemorrhagic illness [DHF] [8]. As per WHO guidelines, thrombocytopenia can be used as a simple diagnostic criteria for DHF [9]. The only accessory laboratory test which supports the diagnosis of dengue is platelet count and it can be roughly estimated by microscopy even in the peripheral laboratories [10]. Hence the present study is designed to correlate the dengue serological markers with platelet count which, not only helps in identifying and categorizing the patient but also in planning management accordingly, thereby curtailing further progression of disease to its severe forms and thus increasing positive prognosis.

MATERIAL AND METHODS

Study design

It is Prospective cross sectional study and total size of the population is 426 and duration of this study is 8 months.

Nature of Study population

All Dengue fever suspicious cases defined according to WHO suggested case classification as Dengue fever, Dengue Hemorrhagic fever & Dengue shock syndrome were enrolled into the study. The serum samples comprised of both acute and early convalescent phase depending on the reporting time of the patients. Acute phase serum samples were collected from patients who reported < 5 days and after 5days of fever in whom NS1 ag assay and Ig M MAC capture ELISA was done. Platelet count was recorded in dengue + and –ve.

Inclusion criteria

Febrile patients admitted in medical and pediatric wards of Osmania General Hospital. Patients of all age groups clinically diagnosed as having DF.

Exclusion criteria

Febrile cases with definite source of infection (eg.respiratory, UTI, Meningitis) as per available clinical and Laboratory data. The following patients were excluded, History of bleeding tendency since birth, Patients with thrombocytopenia and no fever, patients with fever of more than 2 weeks duration and other conditions that cause TCP like Autoimmune connective tissue disorders , ITP, Malignancy.

Data collection

Approval of the Institute's Ethics Committee was obtained to carry out the study. Informed consent was obtained from each patient. Information on demographic features and symptoms of the patients were collected by a structured questionnaire.

Sample collection and storage

2-3 ml of venous blood was collected under aseptic precautions in vaccutainers with no additives and kept at room temperature for 30 min to clot. Serum was separated by Centrifugation and transferred to aliquots and stored at -20 until tested.

Laboratory methods

EDTA blood samples were collected and Hematological parameters were detected in all samples using automated cell count analyzer which were confirmed by peripheral blood smear examination. All the acute phase serum samples were detected for NS1 ag using the Commercially available kit (Panbio Dengue Early Elisa Kit) and Ig M capture ELISA (kit obtained from NIV, Pune). Thrombocytopenia detected in both positive and negative serotypes.

Statistical Analysis

Microsoft office 2007 was used to make tables. Results of the study are based on descriptive statistics using mean and percentages.

RESULTS

In the present study, a total 426 samples were analyzed. Out of 426 samples, more number of seropositives 44 (41.4%) were noticed when 106 serum samples were tested using both NS1 Elisa and MAC Elisa. (Table 16). Out of 132 samples that reported with fever < 5 days duration NS1 ag Elisa was done and 35 samples were seropositive accounting to 26.5%.Out of 106 samples with fever between 6-9 days, both assays were performed and 44 (41.4%) were seropositive .Out of 188 samples received with >10 days of fever, only MAC Elisa was done and 68 showed seropositivity rate of 36.1 %. (Table 1).

Table 1 Distribution of Serological markers

Serological marker	Duaration of fever	No .of samples screened	No.of positives
Only NS1ag	<5 days	132	35(26.5%)
NS1 & IgM	6-9 days	106	44(41.4%)
Only IgM ab	>10 days	188	68(36.1%)

In the present study revealed, that there was variation in different serotypes of dengue infection when we were studied in total NS1 Ag positive cases (Table 2).

Table 2 Detection by using NS1 ELISA

Duration of Fever	Samples Screened	Total NS1 positives	DF	DHF/DSS
<5 days	132	35 (26.5%)	34(25.7%)	1 (0.7%)

25.7 % of DF and 0.7 % of DHF were identified using NS1 ag assay (Table 2)

Table 3 Detection by using MAC ELISA

Duration of fever	Samples screened	Total IgM positives	DF	DHF/DSS
>10 days	188	68(36.1%)	63(33.5%)	5(2.7%)

Table 4 Detection by using both the assays

Duration of	Samples screened	NS1 & IgM positives	DF	DHF/DSS
6-9 days	106	44(41.4%)	43(40.5%)	1(0.9%)

Both markers NS1 and Ig M were observed in 44 cases (41.4%) (Table 3 & 4)

Table 5 Detection rate of both the assays in Dengue Positive Samples

NS1 Ag	IgM		Total
	Negative	Positive	
Negative	Nil	28 (26.4%)	28 (26.4%)
Positive	9(8.4%)	7(6.6%)	15(16%)
Total	9(8.4%)	35 (33%)	44(41.5%)

Chi square test = 19.8 ,p value < 0.001

In the present study out of 106 sample, 44 (41.5%) were positive either for NS1 ag or IgM Ab or both. A total of 16 (15%) samples were positive for NS1ag and 33 (35%) were positive for IgM ab including t significantly increased the detection rate to 41.5%. (Table 5) those that were positive by both. Both the assays, performed together on a single serum sample.

Table 6 Platelet count (per mm^3) in seropositives (n=147)

Range	Positive cases	%
< or equal to 20,000	10	7.80
21,000-40,000	28	21.8
41,000-60,000	29	22.6
61,000- 80,000	23	17.9
81,000-1,00,000	38	29.6
Total	128	87.07

Platelet count <1 lac/cumm is considered as Thrombocytopaenia, which was seen in 87% of seropositive cases. (Table 6). Mild TCP observed in 29.6 % of seropositives**.**

Table 7 Association of Platelet count with seronegativity in Dengue infection n= 426.

Range	seronegative	Percentage%
< or equal to 20,000	10	11.6
21,000-40,000	20	23.2
41,000-60,000	19	22.09
61,000- 80,000	21	24.4
81,000-1,00,000	16	18.6
Total	86	30.8

TCP was seen in 30.8% of seronegative cases. (Table 7). More no. of Seronegatives (24.4 %) were observed between 61,000-80,000/cumm Platelet count.

Table 8 Association of Platelet count with seropositivity in Dengue infection n= 426.

Platelet range (/cumm)	Dengue seropositive	Dengue seronegative
<1 lac	129 (87.7%)	86 (30.8%)
>1 lac	18(12.3%)	193 (69.1%)
Total	147 (100%)	279 (100%)

TCP with seropositivity (87.7%) and seronegativity (30.8%) (Table 8).

Table 9 Grading of TCP

Grades (cumm)	Total cases	Percentage%
Mild (<20,000)	124	29.1
Moderate (20-50,000)	70	16.4
Severe 50,000- 1 lac	20	4.6

Mild TCP noticed in highest no.of patients 29.1, followed by Moderate in 16.4% and severe in only 4.6% of cases (Table 9).

Total 10 Association of platelet count with Serological markers

parameter	Total positives	Platelet count	%
NS1	35	32	91.42
NS1& IgM	44	42	95.45
IgM	68	55	80.88
Total	147	129	87.75

Platelet count <1 lac/cumm was associated significantly when both serological markers were detected (95%) than with individual markers NS1 (91%) and IgM (80.7%) (Table 10).

Table 11 Association of platelet counts with NS1 Ag in Dengue infection

Platelet range	NS1 positive	NS1 negative
<1 lac/cumm	32	37
>1 lac/cumm	3	60
Total	35	97

91.4% TCP was noticed in NS1 seropositive (Table 11)

Table 12 Association of platelet count with NS1 &IgM

Platelet range	Positives	Negative
< 1 lac /cumm	42	22
>1 lac/cumm	2	41
Total	44	63

We also observed that TCP was noticed in 95.4% when simultaneous detection of NS1& Ig M done (Table 12).

Table 13 Association of platelet count with only IgM

Platelet count	Positive	Negative
< 1 lac/cumm	55	26
> 1 lac/cumm	13	94

In the present study revealed that, decrease in Platelet count seen in 80.8 % of Ig M Seropositives (Table 13). From these

results finally we concluded that thrombocytopenia more cases were observed during combined NS1 Ag and Ig M detection.

DISCUSSION

In order to provide timely information for the management of patients and early public health control of dengue outbreaks, it is important to establish the diagnosis of acute dengue virus infection during the first few days of manifestation of the clinical symptoms. Dengue infection has symptoms similar to other viral infection like fever, URTI, malaise, anorexia, bodyache etc. The results obtained were analyzed and evaluated. The role of NS1 Ag for early detection of DV infection is currently being evaluated by many investigators, without the requirement of paired sera.[4,8] NS1 Ag circulates uniformly in all serotypes of dengue virus and it circulates at high level during the first few days of illness.[9] NS1 Ag levels varies from 0.04 – 2 µg/ml in acute-phase serum samples, to only 0 .04µg/ml or even less in convalescent phase serum.[6] This is the reason for its higher detection rate in acute phase sera. In our study, NS1 Ag positivity was 26.5% in acute phase sera in study group I (132 samples). IgM positivity in the acute phase sera in this group was only 36.1% (188 samples). Detection of specific IgM by MAC-ELISA is still used as the diagnostic technique for acute infection; its disadvantage being delayed appearance of antibodies from 5-10 days after the onset of illness in case of primary dengue virus infection and 4-5 days after the onset of illness in secondary infections.

The detection rate of IgM increased in convalescent sera and NS1 Ag detection decreased in acute phase sera in early convalescent sera, which were both statistically significant. Similar findings were seen in other studies along with an increase in sensitivity of detection when both the assays were used together in a single sample.[10,11] A statistically significant increase in detection rate was observed when both the assays were performed together in a single sample.

The morbidity and the mortality of DHF can be reduced by early diagnosis, hospitalization and symptomatic care. In our study, results revealed that out of 132 samples that reported with fever < 5 days duration NS1 ag Elisa was done and 35 samples were seropositive accounting to 26.5%. Among them 25.7 % of DF and 0.7 % of DHF were identified using NS1 ag assay. In the present study, DF was noticed in higher no. of patients (95.3%) than DHF which was observed in only 4.7 % of cases. (Table 12). Present study revealed that out of Out of 188 samples received with >10 days of fever, only MAC Elisa was done and 68 showed seropositivity rate of 36.1 %. In total Ig M positive cases 33.5% samples were shown dengue fever and only 2.7% cases showed DHF.

Thrombocytopenia in dengue infections is not an early indicator of severe disease but it helps in predicting the progression of disease. On comparison of platelet count with dengue seropositivity, thrombocytopenia [platelet count less than 1 lakh, as per WHO guidelines for DHF] is seen in more number of dengue positive cases than dengue negative cases and the average platelet count of dengue negative cases were higher than the dengue positive cases. Reduction in platelet count observed in dengue negative cases may be due to other causes like collagen vascular disorders, viral infections other than dengue, drug induced thrombocytopenia etc. In a study conducted by RD Kulkarni *et al* [7] thrombocytopenia was seen in 68.8% of dengue positive cases and whereas Santosh Shivaji rao Tathe *et al*.; [6] reported 81.72% in their study. On taking the different dengue parameters into account, thrombocytopenia is observed in 61[84.7%] out of 72 NS1 antigen positive cases and 12 [57.1%] out of 21 cases positive of antibodies alone showed thrombocytopenia. Similar findings were observed in the study of RD Kulkarni *et al*.; [7].

Platelet count is decreased in other viral conditions other than Dengue-Idiopathic thrombocytopenia. We therefore, tried to correlate platelet counts in cases of fever that tested negative for any of the dengue parameters.3 In 426 cases of fever, in which none of the dengue parameters was positive, we observed different grades in plate count analysis, mild TCP noticed in highest no.of patients 29.1%, followed by Moderate in 16.4% and severe in only 4.6% of cases. In the present study TLC < 4000/cumm was observed in 3.5 % of seropositive cases as represented in table -21, that showed association with only NS1 ag in 13.3%, only IgM ab in 60% and both NS1and IgM in 26.6 % of positive cases. These results correlated with other previous findings done by Ageep AK *et al* and Itoda *et al* showed higher percentage of Leucopaenia in 90% and 71% cases respectively [114,102]. Sanjay kumar mandal *et al* has suggested that Leucopaenia in DF may be due to myelosuppression. The previous findings showed increased hematocrit of >51% was observed in 50% of male patients[55]. In the present study, total seropositives with platelet count < 1 lac/cumm were 87%. Our reults differed from studies conducted by M .Neeraja *et al*, and Saraswathy MP *et al* who reported higher percentage of TCP i.e. more than 95% [76, 98]. Ragini Singh *et al***,** reported higher percentage of TCP 97.1% which they attributed to be due to Oxidative stress induced decrease in platelet count [88].

Association of TCP with Serological markers in which correlation was noted when both markers were tested (95.4%), than with individual markers only NS1 91.4%, only Ig M 80.8 %. In all the above studies, higher rate of TCP is seen when both markers were simultaneously detected which is in concordance with our study.

Earlier finding reported that, more number of cases 29.1% belonged to Mild category of TCP with platelet count between 50,000 – 1 lac /cumm similar to previous study done by C.V Prathyusha *et al*.[66] where 38.2% cases belonged to Mild category[66]. However 46% and 56.2 % cases belonged to Moderate category with Platelet count ranging from 20,000-50,000 /cumm in studies done by Kakarla Thota Kanugolu respectively [99]. 100 % of DHF cases showed TCP when compared to 87.8% DF cases similar to earlier study done by Ritu Karoli *et al* [86].

CONCLUSION

Association of thrombocytopenia in dengue parameter positive cases was highly significant when compared to thrombocytopenia in dengue parameter negative cases. Nonspecific symptoms of dengue infection during its early phase necessitate the need for its differentiation from other febrile illness. Platelet count of dengue positive cases were significantly lower than the dengue negative cases. While correlating dengue parameters with platelet count,

thrombocytopenia was common in NS1 antigen associated cases than with cases positive for antibodies alone. Thrombocytopenia was much observed in secondary dengue infections than the primary infections. In a country like India where most of the hospitals have poor resources, ELISA, viral culture, and PCR cannot be done for the diagnosis of DI, though the sensitivity of these tests is more than ICT. The antibodies take nearly one week to appear in the blood, therefore, antigen detection by the immune chromatographic test is the only means of diagnosis of DI in the first few days of fever, which helps in management of complications like DHF and DSS.

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