Section: Miscellaneous

Original Research Article

CORRELATION OF HAEMATOLOGICAL AND BIOCHEMICAL PAPAMETERS IN CASES OF DENGUE WITH SEVERITY OF ILLNESS

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ABSTRACT

Background: Dengue imposes serious healthcare, economic and social burden. This study was carried out to correlate the haematological and biochemical parameters with the severity of illness and to classify patients according to WHO classification in detecting severe cases. **Materials and Methods:** A retrospective observational study was conducted in the department of Pathology of a tertiary health care center of Mumbai. The study consisted of suspected cases of dengue fever both outpatient and admitted in the hospital over a period of 17 months (sample size= 100). Blood samples were collected from all the patients suspected of dengue fever for complete blood count, platelet count, haematocrit, liver function test, renal function test, prothrombin time. Dengue viral antibody IgG, IgM, relevant NS1antigen and RT-PCR were performed for all the patients. WHO new revised classification (2009) by tropical disease research was used to classify cases into three groups i.e.dengue without warning signs (DWWS), dengue with warning signs (DWS) and severe dengue (SD). **Results:** Fever was universal symptom (100%), other symptoms being vomiting (77%), abdominal pain (58%), myalgia (100%), joint pains (85%), headache (100%) and rash (30%). All these symptoms were maximum in SD and DWS groups. Gum bleeds (14%) and ecchymosis (14%) were the most common bleeding manifestations. Hepatomegaly (14%) and ascites (8%) and pleural effusion (17%) were the signs found in this study and they were commonly seen in SD group. RT PCR was positive in 91% cases and there were 72% cases of primary dengue and 28% cases of secondary dengue. **Conclusion:** The WHO classification has helped to detect the severe dengue cases from the other two groups. The available haematological and biochemical parameters help in early diagnosis and treatment of severe dengue cases preventing morbidity and mortality.

INTRODUCTION

Dengue is an acute mosquitoe borne viral infection transmitted by Aedes mosquitoe. It is estimated to affect about 390 million people per year of which 70% are in the Asian subcontinent.[1] It is caused by four antigenically different serotypes of the virus referred to as DENV-1 to DENV-4.[1] The severity of dengue infection is varied, with mild self limiting infection to severe dengue like (Dengue hemorrhagic fever) DHF and DSS (Dengue shock syndrome) leading to significant mortality and morbidity.[2] Secondary dengue infection and certain viral serotypes are associated with more severe infections.[2,3] The clinical signs and symptoms of dengue are similar to other infections like malaria, leptospirosis, other viral and bacterial infections.[4] Hence laboratory diagnosis is needed by tests such as NS1 Ag, RT PCR, IgM and IgG tests to confrim the diagnosis. Other laboratory tests are done like hematocrit, platelet count, coagulation studies, renal and liver function tests to know the severity of the infection. Based on the clinical and laboratory parameters WHO has classified dengue into Dengue

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Without Warning Signs (DWWS), Dengue with Warning Signs (DWS) and Severe Dengue (SD). In this context, the present study aimed to correlate the biochemical and hematological parameters of patients with dengue fever along with severity of illness according to revised WHO 2009 guidelines and to identify the laboratory markers which can increase the sensitivity of the screening by healthcare profesionals in the most serious cases.

MATERIALS AND METHODS

A retrospective observational study was conducted for a period of 17 months in the department of Pathology of a tertiary health care center of Mumbai. The study consisted of suspected cases of dengue fever both outpatient and admitted in the hospital.

Method of collection of data: A total of 100 NS1 antigen positive cases of dengue irrespective of age and gender fulfilling the conditions for inclusion criteria were selected. A written and informed consent was taken from all the patients. Clinical symptoms and vital parameters like temperature, blood pressure, pulse rate, respiratory rate and bleeding manifestations if any were recorded in data sheet. Number of cases in different months of the year were also noted to look for seasonal variation.

Hess 's capillary fragility test was done in all by inflating the B.P. cuff to a point midway between systolic and diastolic pressure for 5 minutes and the positive test was documented by the appearance of 20 or more petechiae in an area of one inch square (6.25 cm2) just below the cubital fossa.

Blood samples were collected from all the patients suspected of dengue fever for complete blood count (CBC) including peripheral blood smear examination, platelet count, haematocrit, liver function test, renal function test and prothrombin time. Signs of plasma leakage like ascites, pleural effusion if present were noted from clinical records.

Dengue viral antibody tests like IgG, IgM were performed using a commercially available kit for anti dengue immunoglobulins IgG and IgM and and NS1antigen was done by a rapid immunochromatographic immunoassay card test.

Real time RT-PCR was done on all the 100 samples. RT PCR was done by Taqman approach. RNA extraction was done by using the high pure viral RNA Acid kit.

WHO classification (2009) was used to classify cases into three groups i.e. dengue without warning signs (DWWS), dengue with warning signs (DWS) and severe dengue (SD).

Inclusion Criteria

1) All patients belonging to any age group and gender suspected of having clinical features of dengue with NS1 antigen positivity.
2) All patients admitted or outpatient having NS1 antigen positive result and having thrombocytopenia and also with deranged liver enzymes were included in the study.

Exclusion Criteria

1) All patients who had dengue associated with hematological malignancies, chronic thrombocytopenia or any other illness with low platelet count.
2) Patients with chronic kidney and liver diseases and pregnant patients admitted for febrile illness.

Method of Statistical Analysis

- All the statistical evaluation was done using SPSS .16 software.
- Appropriate statistical test was used to analyse the data.

RESULTS

In this study, out of a total 100 cases of dengue, there were 38 cases in the DWWS, 53 cases in DWS and only 9 cases in SD group. There were 56 males and 44 females and the male to female ratio was 1.27:1. The commonest age group affected was 20-30yrs with the mean age being 22.5+_ 9.77 for males and mean age for females was around 25.7+_11.28.

Among the 100 cases, fever, myalgia and headache was seen in all the cases while joint pains was seen in 85% cases. Vomiting was present in a total 77% cases, abdominal pain was seen in total 58% cases. Rash was present in 30% cases. Among the bleeding manifestations gum bleeds was the commonest manifestation (14%) along wiith ecchymosis (14%), followed by haematuria (13%} and epistaxis (13%). These bleeding manifestations were increased more in the patients belonging to the SD and DWS group. They were not seen in the DWWS group and the difference was statistically significant (P<0.005). Maximum cases of dengue were seen in the monsoon season and the number of cases were highest in September 64% cases followed by 26% cases in August and 5% cases in July. Altered sensorium was present in 3% patients of SD group while it was not present in the other two groups. Occurrence of altered sensorium was significantly higher in severe dengue patients which was statistically significant (P value <0.000). A detailed distribution of cases according to the symptoms is given in the table no 1.

Systolic blood pressure < 90 mm hg was seen in 25% cases. Overall petechiae was seen in 18 % cases and positive torniquet test was seen in 32% cases. Pleural effusion was seen in a total of 17% cases and hepatomegaly was seen in 14% cases. Similarly ascites was seen in total eight percent cases. Thrombocytopenia (TCP) was seen in 54% cases. Table no 2 shows distribution of cases according to the signs.

In DWWS group of dengue there was a fall in platelet count on the 3rd day which became normal on day 5. However in DWS group the platelet count reduced to a minimal value on the 3rd day but showed only partial rise on day 5. In the SD group there was severe TCP which was minimal on the 3rd day and continued to remain on the 5th day. Hence TCP was seen in majority cases of dengue in all three groups.

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Degree of TCP correlated with disease severity. However degree of TCP was more in DWS and SD group. The mean platelet count of all three days in three different groups is considered for analysis and mean platelet count on day 3 is lower in the severe group as compared on the day 5. Chi –square was applied to study the association between platelet count and poor prognosis which was statistically significant (P<0.000). Hence TCP can be used as a marker to assess the severity of cases. Refer to table no 3 for the correlation of degree of TCP with dengue groups.

Leucopenia was seen in a total of 58% cases with 60.4% cases in DWS group and 55.6% cases in the SD group, it was marginally higher in the DWWS and SD group though it was not significant. Lymphocytosis with atypical lymphocyte (>45%) occuring more frequently in 22.6% cases in DWS group and with 33.3% cases in the SD group. Total atypical lymphocytosis were seen in 23% cases.

LIVER FUNCTION TESTS: AST (>38µl) was seen in 76 cases, where 90.6% patients were in (DWS) and 77.8% patients in (SD) group. On applying Chi square test p value was 0.001 (statistically significant). ALT (>35ul) was seen in total 60% cases with 69.8% cases in the DWS group and 77.8% cases in the SD group which was statistically significant P=0.005.

Prothrombin time was prolonged in total 13% cases, with 2.6% cases in the DWWS group, in 15.1% cases in DWS group and in 44.4% cases in the SD group and the difference was stastically significant with P-value (0.001). Serum albumin was also lower in total 17% cases of which 55.6% cases were in the SD group compared to DWS and DWWS group which was significant (P=0.005). Table no 4 gives distribution of cases accorrding to the biochemical parameters.

Out of 100 samples, 91 were positive by RT PCR. In DWWS 89.5% patients tested positive by PCR, 90.6% patients tested positive by in DWS group and 100% patients tested positive in SD group. Seventy two patients were diagnosed as primary dengue whereas secondary dengue observed in 28 patients.

Table 1: Distribution of cases according to the symptoms

SYMPTOMS	DWWS		DWS		SD		P-VALUE	TOTAL	
	n =38	%	n=53	%	n=9	%		n=100	%
Fever	38	100	53	100	9	100	0.000	100	100
Myalgia	38	100	53	100	9	100	0.000	100	100
Joint Pains	32	84.21	45	84.90	8	88.88	0.939	85	85
Headache	38	100	53	100	9	100	0.000	100	100
Vomiting	21	55.3	47	88.67	9	100	0.000	77	77
Abdominal pain	2	5.26	48	90.56	8	88.9	0.000	58	58
Rashes	10	26.3	16	30.2	4	44.4	0.565	30	30
Ecchymosis	0	0	10	18.9	4	44.4	0.001	14	14
Haematuria	0	0	9	17.	4	44.4	0.001	13	13
Haematemesis	0	0	8	15.1	3	33.3	0.006	11	11
Epistaxis	0	0	9	17	4	44.4	0.001	13	13
Gum bleeds	0	0	11	20.8	3	33.3	0.004	14	14
Altered Sensorium				0	3	33.3	0.000	3	33.3

Table 2: Distribution of cases according to the signs

SIGNS OBSERVED	TOTAL		DWWS			DWS		SD	
	N	%	N			%		N	
Systolic BP <90 mmhg	25	25	4	10.5	16	30.2	5	55.6	
Petechiae	18	18	2	5.3	11	20.8	5	55.6	
Torniquet Test	32	32	12	31.6	14	26.4	6	66.7	
Pleural Effusion	17	17	0	0	11	20.75	6	66.66	
Hepatomegaly	14	14%	0	0	9	16.98	5	55.55	
Ascites	8	8	0	0	3	5.66	5	55.55	

Table 3: Correlation of degree of Thrombocytopenia with dengue groups

DAY	PLATELET COUNT	DWWS		DWS		SD		TOTAL	
		N	%	N	%	N	%	N	%
1	<50000	0	0	9	17	4	44.4	13	13
3	<50000	0	0	17	32	7	77.8	24	24
5	<50000	0	0	10	18.9	6	66.7	16	16

Table 4: Distribution of cases according to biochemical parameters

PARAMETERS	TOTAL N	%	DWWS N	%	DWS N	%	SD N	%	P-VALUE
AST(>.38µl/L)	76	76	21	55.3	48	90.6	7	77.8	0.000
ALT(>35µl/L)	60	60	17	44.7	37	69.8	6	66.7	0.005

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Prothrombin Time (>14 sec)	13	13	1	2.6	8	15.1	4	44.4	0.001
Serum Albumin (<3 gm/l)	17	17	3	7.9	9	17	5	55.6	0.005

Table 5: Comparison of various symptoms with different studies

Study	Fever	Myalgia	Joint Pain	Vomiting	Abdominal Pain	Rash	Headache	No of Patients
Present Study	100	100	85%	77%	58%	30	100	100
Nazish Butt	100%	65.40%	82%	79.80%	65.38%	85%	38%	104
Pavan Muniraja	90%	35.60%	40%	8%	9.60%	26%	24%	150
CV Prathyusha	100	32.50%	-	42%	58%	28.70%	-	80
Ira Shah	100%			86.60%	48.70%	41		51
Vanamlai D.R.	100%	65	58	42	45	27	85	190

Table 6: Comparison of various signs with different studies

Study	No of Patients	Raised AST	Raised ALT	AST>ALT
Present Study	100	76%	60%	+
Kuo et al	270	93.3%	82.20%	+
Souza et al	1585	63.40%	45%	+
ITtha et al	45	96%	96%	equal
Parkash et al	699	95%	86%	+
Trung et al	644	97%	97%	+

Table 7: comparison of Liver Function Tests with different studies

Studies	Pleural Effusion	Ascites	Hepatomegaly	Tourniquet Test
Present study	17%	8%	14%	32%
Ira Shah et al	30.8	35.80%	97.40%	-
Nazish Butt et al	10.50%	8%	56.70%	
Pavan Muniraja et al	60%	60%	40%	
C.V. Prathuyusha et la	25%	25%	33.75%	42.50%

DISCUSSION

In this study there were 38% cases in the DWWS, 53% cases in DWS and only 9% cases in SD group. In a study at Ahmedabad by Abhinav Jain et al it was found that out of 56 patients enrolled in the study 43 (76%) cases were DWWS,11 (20% cases) were of DWS, and only 2 (3%) cases were of SD.[5]

In a study by Rathakrishnan.A. et al there were more 12.7% cases of DWWS, 77% cases of DWS and only 15 cases of SD.[6] This finding was similar to the present study in which more cases were in the DWS group than the DWWS.

There is a difference in the percentage of cases in different groups because the study population may vary in different studies. In the present study both outpatients and admitted patients were included hence there are more cases of DWS as compared to some other studies. There were 56 males and 44 females with a male female ratio of 1.27:1. In a study by Abhinav Jain et al there were 60% males and 40% females, while Shazia et al have reported 82% males in their study.[5,7] The male preponderance for dengue infection can be attributed due to more outdoor occupation in males which was also seen in our study.

In this study maximum no of cases occurred during the rainy season with 64% cases in September, 26% in August and 5% cases in July. Similarly Shazia et al has documented more cases of dengue in the monsoon period.i.e in October, this is probably because during rainy season due to ambient temperature and humidity conditions are ideal for mosquito breeding and India being a tropical country with heavy rains and failure of public health measures has lead to endemicity of dengue in urban and semi urban areas.[7]

In this study all the patients had complaints of fever, myalgia and headache irrespective of their category, fever was found to be universal symptom and similar findings were found in the studies by Abhinav Jain et al (100%), Nazish Butt et al (100%), Vanamali DR et al (100%).[5,8,9]

Vomiting was present in 77% of cases in this study which was similar to the study by Nazish Butt (79.8%), while in the studies by Vanamali DR (42%), Pavan Muniraja (8%) and CV Prathyusha (42%) it was less.[8-11]

Most of the patients who had abdominal pain (total 58%) were in the DWS and SD group (88.9%) in the present study (P=0.000) similar to the study by Abhinav Jain et al (50%) and Macedo et al (84.1%).[5,12] This indicates that abdominal pain is a warning sign and it is caused due to hepatic involvement as suggested by previous studies. While in study by Rathakrishnan et al abdominal pain was more in the DWS group than the SD group.[6]

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Most cases having rash in this study were in the SD (44.4%) group similar to study by Macedo et al (34.6%) while in study by Abhinav Jain et al rash was found to be more in the DWWS group.[5,12] Rash was more in the DWS group in a study by Rathakrishnan et al.[6] Table no 5 gives comparison of various symptoms with different studies.

In this study bleeding manifestations were seen in total 21% patients. These findings were more compared to other studies by Pavan Muniraja, CV Prathyusha and Raveendra K.R. et al and they were statiscally significant.[10,11,13] However petechiae were more in studies by Pavan Muniraja et al and CV Prathyusha et al (32.5%).[10,11]

Hepatomegaly is a warning sign and is a risk factor for SD and for shock in children as mentioned in study by Ledika et al.[14] Combined presence of abdominal pain, elevated liver enzymes points to the presence of severe dengue infections as mentioned by Kamani Wanigasuriya et al.[15] Maximum patients who had hepatomegaly in our study belonged to the SD group (55.55%) similar to study by Macedo et al (36.4%) and Fabio lima et al, while more in the DWS group than SD group in study by Rathakrishnan et al.[6,1216,]

Ascites was present in eight percent cases in this study which was comparable to the study by Nazish Butt (8%) et al but less when compared to studies by Pavan Muniraja (60%), Ira shah (35.8%) and CV Prathyusha (25%) et al.[8,10,11,17]

Pleural effusion in our study was present in 17% cases which was similar to study by CV Prathyusha (25%) et al.[11] While in studies by Ira Shah (30.8%) et al and Pavan Muniraja (60%) et al it was more.[10,17]

In this study low systolic BP< 90 mmhg was seen (total 25% cases) in which 10.5% patients in DWWS group, in 30.2% patients in DWS group and in 55.6% patients in SD group. The patients who belonged to DWWS group who had low systolic blood pressure were found to have orthostatic hypotension and were malnourished.

In study by Abhinav Jain et al, hypotension was seen in nine percent patients in DWS group and in 100% patients in SD group and in no patients of DWWS group which correlated with this study.[5] Table no 6 comparison of various signs with different studies.

Thrombocytopenia was seen in total 54% cases which was similar to the study by Tahir Jameel et al (62%) but less compared to studies by Ira Shah et al (92.3%), Nazish Butt et al (100%) and CV Prathyusha (85%)et al.[8,11,17,18] In this study we found the degree of TCP correlates with the severity of illness.

Leucopenia in this study was seen in 58% cases similar to the study by Tahir Jameel et al (56.6%) while it was more in studies by C V Prathyusha et al (66%) and Nazish Butt (52.89%) while Ira Shah (23%) documented it less.[8,11,17,18] In this study atypical lymphocytes were 23% cases. In a study by Tahir Jameel et al atypical lymphocytes seen in 93%, while in study by Nazish Butt they were in 21%, which is similar to this study.[8,18]

In this study 76% patients had increased AST and 60% had increased ALT. Raised AST was seen in the studies by Kuo et al (93%), Itha (96%) et al, Parkash (95%) et al and Trung (97%) et al which was more than this study while in study by Souza (63.40%) et al it was less.[19-23] Table no 7 gives comparison of liver function test with different studies.

Prothrombin time was prolonged in 13% cases in this study which was similar to the study by Nazish Butt et al (19%) while in the studies by Ira Shah et al (33%), Tahir Jameel et al (24%) and Shazia et al (74%).[7,8,17,18] Prothrombin time serves as a marker for dengue shock syndrome.

Serum albumin in this study was found to be low in 17% patients with 55.6% cases in SD group, (P=0.005). In another study by Riffat Mehboob et al it was decreased in 54.5% cases which was more compared to our study.[24]

Majority of RT PCR positive patients belonged to the SD group. Concordance in the present study was 91% and discordance was 9%. Nine % cases which tested negative by RT PCR was due to faulty transport of plasma samples to referral laboratory and defective maintainence of cold chain and repeating the tests was a constraint. In a study by Nishant Ahmed et al comparison of NS1 antigen, real time RT PCR and virus isolation for rapid diagnosis was done where sensitivity of NS1 detection was 73.53% and 79.41% for RT PCR.[25] There was 88.7% concordance and 11.3% discordance

In this study 72 cases were of primary dengue and 28 cases were of secondary dengue. In the study by Rathakrishnan et al 60.5% were primary dengue and 38.5% were secondary dengue similar to our study.[6]

CONCLUSION

Dengue is an infectious disease, endemic in metro cities like Mumbai which has propensity to considerable morbidity and mortality. Since, there is no availability of vaccination, management solely depends on early diagnosis and treatment against a background of other infectious diseases that occur in monsoon season. Various microbiological tests are done to confirm the diagnosis and distinguish between primary and secondary dengue. A host of hematological and biochemical parameters can give a clue about the severity of dengue for optimum management. Apart from this, effective vector control should be implemented to prevent epidemics. Future directions include application of prognostic biomarkers, and nanotechnology to improve knowledge of virus host interactions and vaccine development. A coordinated approach of government, communities and international organisations to recognize the severity, magnitude and strong commitment from local to the global level and implementation of research findings is the need of the hour.

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