Original Article

Indian Journal of Pathology: Research and Practice
Volume 6 Number 1, January - March 2017
DOI: http://dx.doi.org/10.21088/ijprp.2278.148X.6117.12

Clinico-Hematological Profile in Dengue: A Tertiary Care Institutional Study

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Abstract

Introduction: Dengue is the most important arthropod borne viral infection of humans. As there is an alarming rise in Dengue Fever and its complications (i.e, Dengue Hemorrhagic Fever/Dengue Shock Syndrome), rapid and accurate diagnosis is of paramount importance. *Aims/Objectives:* 1. To study the prevalence of dengue in different age groups and sex distribution. 2. To study the spectrum of clinical symptomatology in endemic population. 3. To evaluate the clinical, hematological, biochemical, serological and coagulation profile in different stages of confirmed Dengue cases. 4. To correlate clinical, hematological, biochemical, serological and coagulation findings with disease severity and clinical outcome. *Methods:* A prospective study from June 2014 to June 2015 was done among 100 serologically confirmed cases of dengue by rapid immunologic tests. Evaluation of clinical data, hematological and biochemical parameters with peripheral smear study were carried out. *Results:* A total of 100 serologically positive cases were studied with adults (63%) and children (37%). The commonest clinical presentation was fever (100%), headache (80%), retro-orbital pain (48%), vomiting (18%), myalgia (64%), arthralgia (52%), hemorrhagic manifestation (18%) and shock (06%). The Hematological profile observed were decrease in Hemoglobin (62.5%), Hemoconcentration (35%), Leukopenia (57.5%) with presence of atypical lymphocytes (43.7%). and Thrombocytopenia of varying degree (62%). Liver enzymes were elevated in 25 % of cases and markedly elevated APTT level in 18% of cases of DHF/DSS. *Conclusion:* Early and prompt diagnosis by rapid diagnostic kits along with clinical features and serial measurements of hematological and biochemical parameters helps in reducing the morbidity and mortality associated with Dengue.

Keywords: Dengue; Dengue Fever; Dengue Hemorrhagic Fever; Dengue Shock Syndrome; Severe Dengue.

Introduction

Dengue is the most important arthropod borne viral infection of humans. It is transmitted by mosquitoes of the genus Aedes, widely distributed in the sub-tropical and tropical areas of the world [1].

Recent studies estimate that 3.5 billion people are at risk, over 2 million of cases with severe disease and 21,000 deaths. A 30 fold increase in the number of dengue cases over the past 50 years has been recorded with nearly 119 countries endemic for dengue [2].

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(**Received on** 30.11.2016, **Accepted on** 13.12.2016)

In India based on the data of National Vector Disease Control Program (NVBDCP), the number of dengue cases reported in 2013 were 74,454 with 167 deaths. In 2014 Karnataka reported 1766 cases of dengue of which 66 cases were reported from Raichur, which is one of the endemic districts in Karnataka [3].

Dengue virus infection may be asymptomatic or may cause undifferentiated febrile illness (viral Syndrome), dengue fever (DF) or dengue hemorrhagic fever (DHF) including dengue shock syndrome (DSS) [4]. DHF has a mortality rate of 2-5%, when treated, but when left untreated the mortality rate is as high as 50% [1]. The first objective of the World Health Organization(WHO) global strategy (2012-2020) is to reduce dengue mortality to 50% by 2020 [5].

Although the number of dengue cases has shown a steady rise with every passing year, the mortality has reduced. This reduction is probably the result of the

cumulative effect of better patient management, increased diagnostic capacities and better reporting [6]. Rapid and accurate diagnosis is of paramount importance for clinical management, epidemiological surveillance, research and vaccine trials [4].

Among the laboratory methods available for diagnosis of dengue, virus isolation and viral nucleic acid detection provides the most specific tests. However facilities that can support viral culture are expensive and time consuming and are not always available [7].

Early diagnosis can be done based on clinical features, hematological parameters and by rapid diagnostic kits for anti-dengue antibodies which are readily available tool utilized in all public sector hospitals and private labs [8].

The increasing number of cases and deaths in these patients due to clinical and hematological complications prompted us to take up this study and correlate the spectrum of clinical presentations with hematological, biochemical, serological and coagulation parameters. Since there is no immune prophylactic or specific antiviral therapy available, timely and rapid diagnosis plays a vital role in patient management and implementation of control measures which would have a substantial impact on reducing the mortality and morbidity associated with dengue.

Objectives

1. To study the prevalence of dengue in different age groups and sex distribution
2. To study the spectrum of clinical symptomatology in endemic population.
3. To evaluate the clinical, hematological, biochemical, serological and coagulation profile in different stages of confirmed Dengue cases.
4. To correlate clinical, hematological, biochemical, serological and coagulation findings with disease severity and clinical outcome.

Materials and Methods

The present study was conducted at tertiary care centre from July 2014 to July 2015. Clinically suspected cases of dengue with serological confirmation of either dengue specific NS1 antigen assay and / or IgM and / or IgG antibodies were selected. Evaluation of hematological, biochemical, serological and coagulation parameters were carried out. Clinical data were collected from case sheets and patients themselves wherever possible.

Inclusion Criteria

All the cases serologically found to be positive for (Dengue) NS1 antigen/IgM antibody/IgG antibody were included in the study.

Exclusion Criteria

The patients aged less than 5 years and above 70 years were excluded, as there would be non-cooperation from the case subjects. Serological positive cases of dengue which were also positive for other co existent infections like malaria, typhoid etc were excluded.

The present study comprised of a total number of 100 serologically proven cases. Evaluation of hematological parameters was done by collecting 2 ml of venous blood sample in EDTA prefilled tubes. The analysis was done by the automated Hematology analyzer ABX Pentra 60: 5 part Analyzer. Erythrocyte sedimentation rate was measured by Westergren's method and Peripheral smears were studied after staining with Leishman's stain.

For serology a venous blood sample was used. SD Bioline Dengue NS1+ Antibody Combo Card Test Kits were used to detect NS1 antigen, IgM and IgG antibodies and the test results were expressed as positives/negatives for antigen and both antibodies.

For estimation of biochemical parameters like Alanine transaminase(ALT) and Aspartate transaminase (AST) enzyme levels, 3ml of venous blood sample was collected and analyzed by A-15 BIOSYSTEM fully automated analyzer. For coagulation parameters, analysis of Prothrombin time (PT) and Activated partial thromboplastin time (APTT) was done by Philips COA stat 1 coagulometer after collecting 4.5 ml blood in 0.5 ml of citrate.

The cases in the study have been classified as Anemia, Leucopenia, and Thrombocytopenia following the WHO Classification (1997) and subjected to analysis in accordance with the WHO classification of Dengue 1997 and 2009. The results were analyzed by calculating the Chi square and P-value and the inference was classified as not significant (NS), highly significant (HS) and significant (S).

Results

Out of 120 serologically confirmed cases of dengue, this study comprised of 100 cases, as 20 cases of

dengue had coexistent malaria and / enteric fever and were excluded.

In the present study patients ranged from 5yrs to 70 years and mean age among the distribution of cases was 23.13 years. Maximum number of cases were in the age group of 0-10 years (33cases; 33%). Out of 100 cases, females (54 cases; 54%) were the most affected with Dengue infection when compared to males (46 cases; 46%) and male to female ratio was 1:1.17. Among children, males (19 cases; 51.35%) outnumbered females (18 cases; 48.64%) and male to female ratio was 1:0.95.

Table1 shows distribution of dengue cases according to the WHO 1997 case classification.

Based on the clinical data the majority of cases were diagnosed as DF (70 cases; 70%), DHF (24 cases; 24%) followed by DSS (06 cases; 06%).

The mode of presentation in the present study was fever (100 cases;100%), followed by headache (74 cases;74%), myalgia(56 cases; 56%), arthralgia (51cases;51%), retro-orbital pain (48 cases;48%), pain abdomen (25 cases;25%), hepatomegaly(24 cases;24 %), rashes (21cases;21%), vomiting (16 cases;16%), petechiae (14 cases;14%), splenomegaly (12 cases;12%) followed by shock 08(08%), ascites(07cases;07%), pleural effusion (06cases; 06%), and CNS manifestations (02 cases;02%).

Table 2 highlights that clinical symptoms like myalgia, arthralgia, vomiting, pain abdomen, rashes were statistically significant. Among the clinical signs shock and tourniquet test were observed to be highly significant and significant with a p value of 0.001.

Table 3 depicts the distribution of dengue cases based on serology by using NS1+ Antibody Combo Card Test Kits, showed positivity for NS1 antigen in 23 cases (23%). The observation for individual markers like IgG antibody and IgM antibody were statistically highly significant(p-value 0.005) and significant (p-value 0.04) respectively . More than one parameter markers were detectable in the remaining 23% of the cases and were highly significant for combined NS1+IgM and NS1+IgM+IgG with a p value < 0.01.

Table 4 shows interpretation of all hematological parameters. Among all anemia was seen in (39 cases; 55.71%) of DF, (07 cases; 29.16%) of DHF and (02 cases; 33.33%) of DSS cases, and is statistically significant with a p-value of <0.05. Hematocrit levels were raised in (36 cases; 36%) cases and found to be decreased in (29 cases; 29%) and normal in (35 cases; 35%) of the cases. Hemoconcentration was seen in DF (06 cases; 8.57%), DHF(24 cases; 100%) and DSS(06 cases; 100%) and was highly significant with a p-value of <0.001.

Leucopenia as an important observation was found to be statistically significant (p-value of 0.01) among DF (24 cases; 34.29%), DHF (16 cases; 66.66%), and DSS (04 cases; 66.66%).

Absolute neutrophilic count ranged from 380 to17,248 cells/cumm with a mean value 3198.22 cells/ cumm. Neutropenia was observed in DF (09 cases; 12.87%) cases, DHF (14 cases; 58.33%) and DSS (03 cases; 50%), was highly significant with a p-value of <0.01.Among 44 cases of leucopenia, relative lymphocytosis was observed in (21 cases; 21%) with p-value of 0.05 .

Thrombocytopenia as one of the important marker of mortality and disease outcome was seen DF (34 cases; 48.57%), DHF (22 cases; 91.6%) and DSS (06 cases; 100%).The correlation of the clinical spectrum of dengue with thrombocytopenia was highly significant with p value of <0.01.

Peripheral smear examination showed predominantly atypical lymphocytes among (44 cases; 44%) and various other features like giant platelets (11 cases; 11%), normoblasts (07cases; 07%) band forms and shift to left in (04 cases; 04 %) and toxic granules (03 cases; 03 %) .

Among serum Aminotransferases , ALT levels ranged from 11-1560 IU/L and were elevated in DF (11 cases; 15.71 %), DHF(14 cases; 58.33%) and DSS (06 cases; 100%) and AST levels ranged from 12-1304 IU/L, were elevated in DF(03 cases; 4.28%), DHF(13 cases; 54.16%) and DSS (06 cases; 100%). The observed values were statistically significant with p-value of <0.05.APTT was increased in DHF (11cases; 46%) and all cases of DSS (06 cases; 100%).APTT levels are highly significant in cases of DSS.

Table 5 shows disease outcome and its relation to thrombocytopenia. In the present study thrombocytopenia was observed in (62 cases; 62 %), out of which (57cases; 57%) recovered and (02cases; 02%) expired. The cases presenting with platelet levels more than 1,00,000 c/cumm were (38 cases; 38%), out of which (35 cases; 35%) recovered and (03 cases; 03 %) discharged.

In Table 6 an attempt was made to classify dengue cases using WHO 2009 Dengue case Classification according to the levels of severity and the comparison was done with 1997 WHO Dengue classification. As per 2009 WHO classification of severity, (38 cases; 38%) were classified as Dengue without warning signs, (52 cases; 52%) under Dengue with warning signs and (10c ases;10%) as Severe Dengue. With application of WHO 2009 classification, 32 cases

which were previously diagnosed as DF were regrouped under Dengue with warning signs and 04 cases of DHF were upgraded as severe Dengue.

Table 1: Distribution of various presentation of dengue

Final Diagnosis	Cases	Percentage (%)
DF	70	70
DHF	24	24
DSS	06	06
TOTAL	100	100

Table 2: Depicting the analysis of various symptoms

Clinical presentation	DF		DHF		DSS		Chi square	p-value	Inference
	No	%	No	%	No	%			
Fever	70	100	24	100	06	100	3.1	0.212	NS
Headache	47	67.14	21	87.5	06	100	2.09	0.351	NS
Myalgia	48	68.57	04	70.83	02	33.33	10.58	0.005	Sig
Arthralgia	45	62.85	04	16.66	02	33.33	9.3256	0.009	Sig
Retro-orbital pain	33	47.14	12	50	03	50	0.0912	0.95	NS
Vomiting	12	17.14	12	50	02	33.33	8.68	0.01	Sig
Pain abdomen	11	15.71	11	45.83	03	50	8.92	0.011	Sig
Rash	08	11.42	11	45.83	02	33.33	11.73	0.002	Sig
Tourniquet test	14	20	11	45.83	06	100	5.79	0.05	Sig
Petechiae	03	4.28	06	25	05	83.33	0.568	0.75	NS
Hepatomegaly	11	15.71	09	37.5	05	83.33	3.75	0.15	NS
Splenomegaly	04	5.71	09	37.5	05	83.33	0.63	0.73	NS
Shock	0	0	02	8.33	06	100	9.47	0.008	HS
Pleural effusion	0	0	04	16.66	02	33.33	2.76	0.2516	NS
Ascites	0	0	05	20.83	02	33.33	3.65	0.16	NS
CNS manifestation	0	0	001	4.16	01	16.66	0.93	0.63	NS

S-not significant, H-highly significant, S-significant

Table 3: Distribution of cases based on serology

TEST	DF		DHF		DSS		Chi square	p-value	Inference
	No	%	No	%	No	%			
NS1	20	21.42	03	12.5	00	00	4.5	0.1	NS
IgM	12	22.85	09	12.5	00	00	6.16	0.04	Sig
IgG	30	12.87	03	37.5	00	00	10.6	0.005	HS
NS1+IgM	2	17.14	04	16.66	02	33.33	10.2	0.006	HS
IgM+IgG	6	15.71	05	20.83	03	50	4.29	0.11	NS
NS1+IgM+IgG	0	10	00	00	01	16.66	15.82	0.0003	HS
TOTAL	70	100	24	100	06	100			

HS: Highly Significant; NS: Not Significant; Sig: Significant

Table 4: Depicting the laboratory parameters in dengue as per 1997 WHO revised classification.

Laboratory parameters	Dengue Fever (70)		Dengue Hemorrhagic Fever (24)		Dengue Shock Syndrome (06)		Chi square	p-value	Inference
	CASES	%	CASES	%	CASES	%			
ANEMIA	40	55.71	07	29.16	02	33.33	6.22	0.04	Sig
HCT>40 %	06	8.57	24	100	06	100	67.5	<0.0001	Sig
LEUKOPENIA <4,000 c/cumm	24	34.29	16	66.66	04	66.66	8.94	0.01	Sig
THROMBOCYTOPENIA <1,00,000 c/cumm	34	48.57	22	91.66	06	100	6.84	0.03	HS
ALT> 35IU/L	11	15.71	14	58.03	06	100	9.58	<0.05	Sig
AST> 38 IU/L	03	4.28	13	54.16	06	100			
APTT>40 sec.	0	0	11	64.70	06	35.29	7.03	1	NS

HS: Highly Significant;NS: Not Significant; Sig: Significant

Table 5: Depicting the outcome based on platelet count

Final Diagnosis	Outcome			Total
	Recovered	Discharged	Expired	
<1,00,000	57	03	02	62
>1,00,000	35	03	00	38
TOTAL	92	06	02	100

Table 6: Showing the Comparison of dengue 1997 classification along with the revised 2009 dengue case classification according to levels of severity

Classification	Dengue without warning signs		Dengue with warning signs		Severe Dengue	
	Cases	Percentage	Cases	Percentage	Cases	Percentage
DF (70cases)	38	54.28	32	45.71	-	
DHF (24 cases)	-	-	20	87.5	04	16.66
DSS (06 cases)	-	-	-	-	06	100

Discussion

Although vector control programs are launched in endemic areas, India being a tropical country factors like change in climate, urbanization, poor living conditions and inadequate waste management, vector borne disease like dengue fever are showing increase in trend of disease burden.

Children are at higher risk as it has been suggested that base line microvascular permeability is greater than that of adults and this could partly explain why DHF is more frequent in children.

Banerjee et al, Prathyusha et al, Samantha et al showed common age group affected as 10-12 years. The risk factors associated with female gender like pregnancy, obesity, diabetes may explain the female preponderance in adults in our study [9-11,14].

Studies have demonstrated overlap between case definitions of DF, DHF and DSS supporting the concept of dengue as a continuous spectrum of disease rather than distinct entities. Studies by Kumar et al and Sri Rezeki et al showed more number of DF cases (83.91 and 67.1%) than DHF (8.8% and 14.71%) and DSS (7.3% and 4.5%) respectively which correlated with our study [12,13]. Clinical profile in this study showed that all cases presented with fever (100%) followed by headache, myalgia and arthralgia. Singh et al showed rash was typically macular or maculopapular, often confluent in 20% cases. Banerjee et al observed hepatomegaly in 15% cases and was common finding in patients with secondary infection associated with increased transaminases. Malvige et al showed CNS manifestations in the form of restlessness, drowsiness, altered sensorium and encephalitis in 6.8% cases and shock in 12.46% cases almost comparable to our study [9,14,15].

Immunity to single dengue virus (DENV) infection does not provide heterologous immunity to subsequent infection. Study by Gibbons et al, 1.2% of total dengue admitted were found to be repeat infections and reported that this prevalence was 0.5% over the previous 15 years and suggested that incidence of repeat dengue infections increasing throught the world. According to Dutta et al NS1positivity of 23.3% and IgG positivity of 40.28% were comparable, whereas IgM positivity was comparable with Kularatne et al (IgM 18%), combined NS1 and IgM were comparable with Kulkarni et al (11%) [16-18]. In the present study majority of cases showed normocytic normochromic anemia followed by microcytic hypochromic anemia with hemoglobin levels(3.6-19 gm/dl), was observed among 48% of cases which were correlating with study done by Benerjee et al showing 51.85% cases with anemia [9].

Rise in hematocrit is an important warning sign which occurred in the late stage of Dengue viral infection and is unique for DHF, before onset of shock. Prathyusha et al found 41.25% of cases showing increased hematocrit and observed that rising hematocrit being related to disease severity in DHF and DSS [19].

Towards the end of febrile phase there is reduction in number of total leukocytes and neutrophils with relative increase in lymphocytes with appearance of atypical lymphocytes. This observation is valuable in predicting end of febrile period and beginning of critical phase. Arif et al and Ahmad et al showed leucopenia in 43% and 43% respectively and Lieu et al showed presence of atypical lymphocytes in 49% cases and were correlating with the present study [19-22].

Thrombocytopenia and dysfunctional platelets remain a central hallmark of dengue fever. A platelet count of ≤ 1 lak / cumm is usually found between the days 3 and 8 of illness. Thrombocytopenia is most likely due to increased peripheral immune destruction and consumption mediated by complement activation

with binding of platelets to C_3 and viral antigen. Kulkarni et al and Malvige et al showed thrombocytopenia 68.75% and 70.19% respectively [15,17,20]. The Dengue virus may provoke varying degrees of damage to the hepatic parenchyma, ranging from mild increase in aminotransferases to increase upto 30 times the reference values. Therefore the use of liver enzymes ALT and AST to evaluate the degree of liver damage is of great importance. The levels of raised ALT(31 cases; 31%) and AST (22 cases; 22 %) were comparable to the study done by Souza et al with raised levels of ALT in 32.1 % and AST in 43.9 % of cases respectively. With these observations it can be inferred that in patients with DHF the levels of ALT and AST were significantly raised compared to DF cases. In the present study the APTT was raised in (17cases:17%) , comparable with study of Irfan arshad et al, (28.3%) [21,23].

Two cases of dengue succumbed to the illness, one due to encephalitis aged 04years male and the other a female aged 22years. Both had platelet count below 1 lakh/cumm.

The findings of the DENCO study evaluating the 1997 case definitions formed the basis of the revised 2009 WHO case definitions, which classified the illness into dengue with and without warning signs and severe dengue. In this study 32 cases of DF when classified as per WHO 2009 classification fell into the category of dengue with warning signs, showing risk of under diagnosing the severity of dengue disease, if classified according to WHO 1997 classification [13].

Although the revised scheme is more sensitive to the diagnosis of severe dengue and beneficial to triage and case management, there remains issue with its applicability. It is considered by many to be broad, requiring more specific definition of warning signs. This study has attempted to identify clinical and laboratory features of dengue fever in a simpler way and with correlation that will be useful for diagnosing the different presentation of dengue.

Key Message

Dengue is now one of the most important tropical diseases. While it doesn't kill that many people, it has tremendous economic and social impact.

Conclusion

There were large number of reports on atypical clinical presentations of dengue, but they are not backed up by virological studies due to lack of such facilities in most of the hospitals. India needs a large number of virus laboratories that may provide quick and reliable diagnosis. The ELISA based serological, molecular and virological methods are tedious process in terms of time, technique and cost effectiveness.

Pronounced hematological, biochemical and coagulation changes are associated with the disease severity and play an important role in recognizing the illness in the early stage and these would help in taking appropriate steps to modify the outcome of the disease.

Hence to know the severity of this dreaded disease, WHO (2009) classification needs to be advocated for future research on the subject, which is more simplified and has an edge in categorizing the dengue illness, compared to the previous WHO (1997) classification.

As this arthropod borne disease is on the rise globally, a correlation with clinical, hematological, biochemical, serological and coagulation profile will enhance the diagnosis of dengue with effective patient management and favorable clinical outcome.

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