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Haematological Profile in Different Clinical Spectrums of Dengue.

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ABSTRACT

Background: To describe the hematological profile occurring at different clinical spectrums of dengue. **Methods:** Study was conducted in Department of Pathology, Teerthankar Mahaveer Medical College and Research Centre Moradabad, Uttar Pradesh. A total of 100 cases were enrolled with patients who were serologically positive for Dengue either by ELISA/Card(NS1, IgG, IgM) method. A blood specimen of 2 ml was obtained from all the patients under aseptic conditions and was sent for evaluation using Sysmex XS 800i automated counter. Various hematological and biochemical parameters were noted. All the patients were followed up till day 5. **Results:** Out of a total of 100 cases enrolled in the study a total of 55 (55%) did not have bleeding manifestations and comprised the Group I of study whereas remaining 45 (45%) patients presented with bleeding manifestations and were placed in Group II of study. Age of patients ranged from 4 to 80 years. Maximum number of patients were in age group 21 to 40 years (42%). Mean age of patients was 34.04±17.58 years. Majority of cases were detected on ELISA (54%). Mean TLC was 6549±4093/cumm. A total of 87% cases had platelet count <150,000/cumm. Group I where 34.5% patients had platelet count <50,000 in Group II this proportion was 62.2%, thus showing a significant difference between two groups (p=0.038). The proportion of patients with platelet count >100,000/cumm was 26%, 22% and 35% respectively on baseline, day 3 and day 5 where as proportion of those with platelet count <20,000/cumm was 16%, 10% and 6% respectively on baseline, day 3 and day 5 respectively. No significant difference between two groups with respect to any of the biochemical parameters (p>0.05) was observed. **Conclusion:** The findings in present study provide a deep insight into the hematological picture vis-à-vis the clinical and biochemical profile of dengue fever at a tertiary care centre. Considering the relevance of onset and hematological changes, the findings in present study were somewhat skewed as most of the patients included in the present study had delayed admission and as such the onset record was not available. The present study was also limited by the duration of follow-up and outcome evaluation, including relapse.

Keywords: Dengue, Haematological parameters, Biochemical profile.

INTRODUCTION

The origin of the Spanish word dengue is not certain, but it is possibly derived from dinga in the Swahili phrase Ka-dinga pepo, which describes the disease as being caused by an evil spirit and is generally interpreted as fever with hemorrhage.[1] Dengue Fever (DF) is a benign syndrome caused by several arthropod – borne viruses, which is characterized by high grade fever, myalgia, or arthralgia, rash, leukopenia and lymphadenopathy. It is also known as 'break bone fever'. In a small proportion of cases the disease develops into the life- threatening dengue hemorrhagic fever, resulting in bleeding, low levels of blood platelets and blood plasma leakage, or into dengue shock syndrome, where dangerously low blood pressure occurs.[2]

Dengue fever is the most common arthropod borne viral disease. Dengue fever is one of the most important emerging diseases of the tropical and sub tropical regions, affecting urban and pre urban areas and rural areas.[3] Dengue / DHF are widely prevalent in India and all the 4 serotypes(DEN-1 , DEN-2, DEN-3, DEN-4) are found also referred to an arbovirus that belongs to the genus Flavivirus of the family Flaviviridae.[4,5] Transmission to humans occurs by the bite of female Aedes aegypti mosquito infected by one of the four serotypes of the virus. The period of transmission from humans to mosquito begins in one day before the start of fever to upto the sixth day of illness corresponding to the viremia phase. After the female bites an individual in the viremia phase, viral replication (extrinsic incubation) begins in the vector from eight to twelve

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day. In humans incubation time is between 3 to 15 days with an average of 5 days.[6,7] Clinical, epidemiological and laboratory investigations play an important role in the diagnosis of dengue fever. Although hematological investigations like blood count count, platelet count, prothrombin time (PT), activated partial thromboplastin time and tourniquet test are less specific yet helpful in guiding the diagnosis and management of the patient. Evaluation of liver function tests and serum albumin levels also help in diagnosis and management to some extent.[8,9] Some of the other hematological changes include leucopenia, which sometimes reaches to the counts as low as 2x103µL. Mild leukocytosis with neutrophilia is often a common finding at the onset of the disease while lymphocytosis which presents with atypical lymphocytes. Interestingly, hematocrit concentration shows a variability with the duration of illness that leads to progression towards DHF.[10,11]

The present study was carried out with an aim to describe the hematological profile in different clinical spectrums of dengue.

MATERIALS AND METHODS

A 1 year retrospective study was conducted in Department of Pathology, Teerthankar Mahaveer Medical College and Research Centre Moradabad, Uttar Pradesh. A total of 100 cases were enrolled with patients of all age groups, and those who were serologically positive for Dengue either by ELISA/Card (NS1, IgG, IgM) method were included. On the other hand patients found to be serologically dengue negative serologically positive patients who were also found to be positive for other coexisting infections, viz. malaria, typhoid, etc and not completing all the investigations were excluded. A blood specimen of 2 ml was obtained from all the patients under aseptic conditions and was sent for evaluation using Sysmex XS 800i automated counter. The following hematological parameters were noted: Haemoglobin (Hb),Total leukocyte count (TLC), Hematocrit (Hct), Platelet count (PC), Prothrombin time (PT), Activated Partial Thromboplastin Time (APTT).Following biochemical parameters were also assessed: Aspartate aminotransferase(AST),Alanine aminotransferase (ALT), Albumin, Alkaline phosphatase (ALP). All the patients were followed up till day 5. A repeat blood sample was taken at day 3 and day 5 after admission for platelet count evaluation.

RESULTS

Out of a total of 100 cases enrolled in the study a total of 55 (55%) did not have bleeding manifestations and comprised the Group I of study whereas remaining 45 (45%) patients presented with bleeding manifestations and were placed in Group II of study.

Age of patients ranged from 4 to 80 years. The distribution of cases is described below, on comparing the two groups statistically, the difference was not found to be significant statistically (p=0.917). [Table1].

Table 1: Age Distribution of cases.

SN	Age Group	Total (n=100)	Group I (n=55)		Group II (n=45)	
			No.	%	No.	%
1	≤ 10 Years	8	5	9.1	3	6.7
2	11-20 Years	20	10	18.2	10	22.2
3	21-30 Years	20	12	21.8	8	17.8
4	31-40 Years	22	11	20.0	11	24.4
5	41-50 Years	11	7	12.7	4	8.9
6	51-60 Years	14	7	12.7	7	15.6
7	61-70 Years	2	2	3.6	0	0.0
8	71-80 Years	3	1	1.8	2	4.4
Mean Age±SD (Range) in Years		34.04±17.58 (4-80)	33.87±17.08 (6-75)		34.24±18.37 (4-80)	

't'=0.105; p=0.917 (NS)

Majority of cases were detected on ELISA (54%). Among different ELISA methods, ELISA NS1 detected 32 cases, ELISA IgG 8 and ELISA IgM 12. There were 2 cases who were positive for ELISA NS1 as well as ELISA IgG.Card method was used in 46% cases. A total of 32 cases were positive for NS1, 8 IgM and 5 IgG. There was 1 case who was positive for both NS1 and IgG. [Figure1].

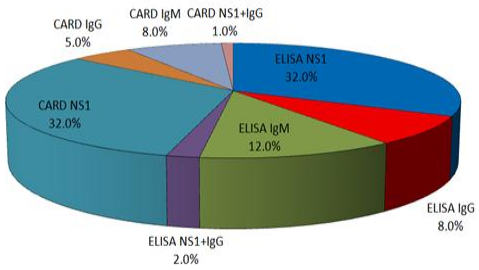

Figure 1: Distribution of cases according to method of detection.

Clinically, fever was the most common complaint seen in all the patients. Body ache was the next most common complaint (64%) followed by vomiting (48%), bleeding (45%), hepatomegaly (40%), rashes (36%) and petechie by tourniquet test (29%) respectively.On comparing the two groups, statistically, no significant difference was observed with respect to presence of fever, rashes, bodyache, vomiting and tourniquet. However, incidence of hepatomegaly and bleeding was significantly higher in Group II as compared to that in Group I ($p<0.001$). In fact, all the 45 cases with bleeding manifestation were in Group II as it was the criteria for differentiation between two groups. [Table 2]

Table 2: Distribution of cases according to Clinical Presentation.

SN	Finding	Total (n=100)	Group I (n=55)		Group II (n=45)		Statistical significance	
			No.	%	No.	%	$\chi 2$	'p'
1	Fever	100	55	100	45	100	-	-
2	Rashes	36	21	38.2	15	33.3	0.253	0.615
3.	Hepatomegaly	40	14	25.4	26	57.8	10.77	0.001
4.	Bodyache	64	34	61.8	30	66.7	0.253	0.615
5.	Vomiting	48	26	47.3	22	48.9	0.026	0.872
6.	Bleeding	45	0	0	45	100	100	<0.001
7.	Tourniquet	29	16	29.1	13	28.9	0.00	0.982

Overall, majority of cases (51%) had haemoglobin levels >12 g/dL followed by those having Hb levels in 10-12 g/dL (33%), 8-10 g/dL (11%) and <8 g/dL (5%) range. Mean Hb levels were 12.43±2.56 g/dL. In Group I, mean Hb levels were 12.03±2.22 and in Group II, mean Hb levels were 12.91±2.87 g/dL respectively. On comparing the two groups the difference was not found to be significant statistically ($p>0.05$). [Table 3]

TLC levels were below 4000/cumm in 24%, in 4000-11000 range in 63% and >11000/cumm in 13% patients. Mean TLC was 6549±4093/cumm. In Group I mean value was 6347±4000 as compared to 6796±4235/cumm in Group II. However, the difference between two groups was not significant statistically ($p>0.05$). [Table 3]

Overall, there were 71% patients with hematocrit >35%. In Group I this proportion was 72.7% whereas in Group II, this proportion was 68.9%. Overall mean Hct levelsw were 38.4±7.4%. In group I, mean Hct was 37.4±6.4% and in Group II, it was 39.7+8.3%. Statistically, the difference between two groups was not significant ($p>0.05$). [Table 3]

A total of 87% cases had platelet count <150,000/cumm. There were 31% patients having platelet count in 20000-50000/cumm range followed by those having platelet count in 5000-100000/cumm range (27%), <20000/cumm range (16%) and 10000-150000 and >150000 range (13% each). As compared to Group I where 34.5% patients had platelet count <50,000 in Group II this proportion was 62.2%, thus showing a significant difference between two groups ($p=0.038$). Overall mean platelet count was 75+72 thousands/cumm. The mean platelet count was higher in Group I (90+75 thousands/cumm) as compared to that in Group II (56+64 thousands/cumm). Statistically, this difference was significant too ($p=0.038$). [Table 3]

Prothrombin time was >14 s in 56% cases. In Group I, 50.9% patients had PT>14s whereas in Group II, 62.2% had PT>14 s. Though proportion of those with PT >14s was higher in Group II as compared to that in Group I yet this difference was not significant statistically ($p=0.256$). Overall, mean PT was 14.8+0.99s. It was slightly lower in Group I (14.76±1.05s) as compared to that in Group II (14.93+0.92s) but this difference was not significant statistically ($p=0.398$). [Table 3]

Activated partial thromboplastin time was >28 s in 54% cases. Although relatively higher proportion of patients in Group II (60%) as compared to those in Group I (49.1%) had APTT>28s yet this difference was not significant statistically ($p=0.276$). Overall, mean APTT was 29.15+1.22s. It was slightly higher in Group II (29.20+0.99s) as compared to that in Group I (29.11+1.38s) but this difference was not significant statistically ($p=0.712$). [Table 3]

A total of 77% patients had AST >40 IU/L and 80% had ALT>35 IU/L There were 57% cases having S. albumin level <3.5 g/dL. A total of 2% had S. ALP <44 IU/L, 92% had S. ALP in 44-147 IU/L and 6% had S. ALP >147 IU/L category. Statistically, there was no significant difference between two groups with respect to any of the biochemical parameters ($p>0.05$). [Figure 2]

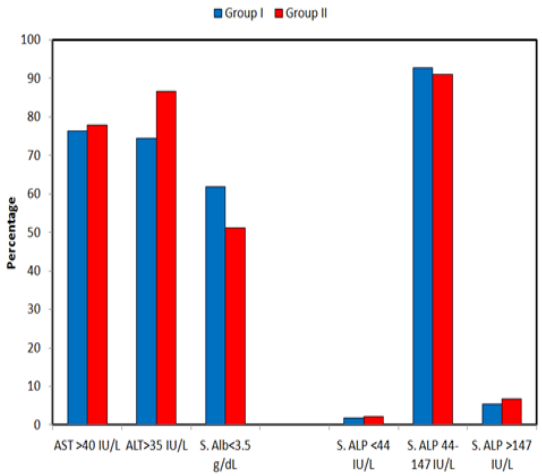

Figure 2: Comparison of Biochemical Parameters between two groups.

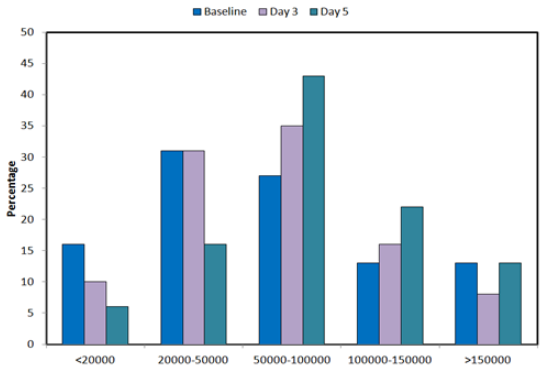

Figure 3: Platelet count levels at baseline and different follow up-intervals.

Proportion of patients with platelet count >100,000/cumm was 26%, 22% and 35% respectively on baseline, day 3 and day 5 where as proportion of those with platelet count <20,000/cumm was 16%, 10% and 6% respectively on baseline, day 3 and day 5 respectively. Mean platelet count was 75±72 thousand/cumm, 71±53 thousand/cumm and 92±54 thousand/cumm respectively at baseline, day 3 and day 5 respectively. On comparing the change in platelet count statistically, it was found to be significant at day 5 only (p=0.004). [Figure 3]

Table 3: Hematological Profile of Cases.

SN	Finding	Total (n=100)	Group I (n=55)		Group II (n=45)		Statistical significance	
			No.	%	No.	%	χ2	'p'
1	Hb level							
	<8 g/dL	5	4	7.3	1	2.2		
	8-10 g/dL	11	7	12.7	4	8.9		
	10-12 g/dL	33	19	34.5	14	31.1		
	>12 g/dL	51	25	45.5	26	57.8	2.42	0.490
	Mean Hb±SD (g/dL	12.43±2.56	12.03±2.22		12.91±2.87		't'=1.737; p=0.085 (NS)	
2.	TLC (/cumm)							
	<4000	24	13	23.6	11	24.4		
	4000-11000	63	37	67.3	26	57.8		
	>11000	13	5	9.1	8	17.8	1.798	0.407
	Mean TLC±SD (/cumm)	6549±4093	6347±4000		6796±4235		't'=0.544; p=0.587	
3.	Hematocrit >35%	71	40	72.7	31	68.9	0.177	0.674
	Mean Hct±SD (%)	38.4±7.4	37.4±6.4		39.7±8.3		't'=1.566; p=0.121	
4.	Platelet count (/cumm)							
	<20000	16	8	14.5	8	17.8		
	20000-50000	31	11	20.0	20	44.4		
	50000-100000	27	16	29.1	11	24.4		
	100000-150000	13	10	18.2	3	6.7		
	>150000	13	10	18.2	3	6.7	10.179	0.038
	Mean PC±SD ('000)	75±72	90±75		56±64		't'=2.378; p=0.019	
5.	PT>14s	56	28	50.9	28	62.2	1.286	0.256
	Mean PT±SD (s)	14.8±0.99	14.76±1.05		14.93±0.92		't'=0.850; p=0.398	
6.	APTT>28s	54	27	49.1	27	60.0	1.186	0.276
	Mean APTT±SD (s)	29.15±1.22	29.11±1.38		29.20±0.99		't'=0.370; p=0.712	

DISCUSSION

Dengue has become a seasonal epidemic that affects the North India almost every year with variable intensity. Clinically, dengue is ill-differentiated from other causes of acute fever viz. malaria and typhoid, however, management of dengue fever requires different approach. In case of improper management, dengue fever often complicates into dengue hemorrhagic fever and dengue shock syndrome, which are associated with poorer outcomes. Hematological parameters have been shown to provide some useful information regarding dengue severity and have been shown to correlate with the clinical spectrum and outcome. Hence, it is essential that the relationship between hematological parameters and clinical spectrum of dengue fever is properly understood.Thus, the present study was taken up in order to describe the hematological profile in different clinical spectrums of dengue.

Out of a total of 100 serologically proven cases of dengue fever, 45 cases (45%) had bleeding manifestations during the course of study. The prevalence of bleeding manifestations among dengue patients has been shown to vary substantially in different studies. Butt et al.[12] in their study reported bleeding manifestations in 34.6% patients while Vanamali et al.[13] reported them in 32% of their series of dengue patients. Kauser et al.[14] in their series reported bleeding manifestation in only 9.58% of cases while Khan et al.[15] Reported them in 19.3% patients. Thus, all these studies reported bleeding manifestations in relatively lesser proportion of cases than in present study. However, researchers like Azin et al.[16] reported the presence of bleeding manifestations in as many as 90 out of 154 (58.4%) of their patients. The findings of present study are close to the findings of Karoli et al.[17] who recorded bleeding manifestations in 40% of their patients. In fact, the bleeding manifestations in

dengue fever might vary depending on the severity of dengue fever and its progression.

In present study, age of patients ranged from 4 to 80 years. Mean age of patients was 34.04±17.58 years. Majority of patients were in age group 11 to 40 years (62%). These findings indicate that dengue virus affects almost all the age groups, however, more particularly in younger age group. However, the proportional distribution of patients across different age groups varies substantially in different studies. Azin et al.[16] reported a study in which 42.9% patients were aged <15 years. However, in the study of Singh et al.[18] half the patients were in 12-30 years age group while Kauser et al.[14] found age group 18-30 years to be the dominant affected age group (68.49%). In present study too, age group 11-20 years had 40% of total cases. The affected age group in different study might be variable owing to difference in sampling frame based on age, for example Unnikrishnan et al.[19] in their study included only elderly patients above 60 years of age and reported the mean age of patients as 66.1±4.7 years. On the other hand, there are studies specifically performed in pediatric age group. Nagaram et al.[20] and Sharma et al.[21] performed their studies exclusively amongst children and reported the mean age of patients to be less than 10 years. However, workers like Patel et al. (2016),[22] similar to present study did not impose any age restriction and reported the age range of patients from 1 to 76 years. In present study, we did not find a significant difference in age of patients with and without bleeding manifestations.

In present study, male to female ratio was almost equal with 51% males and 49% females. However, other studies generally show a dominance of males. Kauser et al.[14] in their study had 63.0% males while Patel had 67% males.[22] Proportion of males above 60% of study population has also been reported by other workers too. But Shekhar et al.[23] in their study had gender profile similar to that observed in present study with 53% males and 47% females. Nagaram et al.[20] also had 54.6% male patients as compared to 45.4% females. There is as such no physiological or epidemiological reason for a gender-bias, in fact, both males and females are equally susceptible to dengue virus fever.

In present study, we used both ELISA as well as Card methods to detect the dengue virus. For both the methods DENV non-structural 1 (NS1) protein antigen was most successful in diagnosis (64%). Dengue NS1 antigen, a highly conserved glycoprotein, produced in both membrane-associated and secretion forms, is abundant in the serum of patients during the early stages of DENV infection. However, a number of cases in present study presented after a crucial delay. In such cases, IgG and IgM antibody diagnosis plays a crucial role. In present study we were able to diagnose 36% cases using IgM/IgG antibody detection methods.

In present study, fever (100%), body ache (64%), vomiting (48%), bleeding (45%), hepatomegaly (40%), rashes (36%) and tourniquet (29%) were the most common presenting complaints. Among patients with bleeding manifestations, incidence of hepatomegaly was significantly higher as compared to that in patients without bleeding manifestations. Dominance of clinical features like bodyache, vomiting, rashes and bleeding has been shown in a number of other studies too, however, following fever, the most common presenting complaints varies in different studies. Butt et al.[12] in their study reported rashes (81.7%) as the second most dominating feature after fever, Hasan et al.24 reported vomiting (55.6%), Unnikrishnan et al.[19] and Vulavala et al.25 reported myalgia (43.4% and 94.83%) and Sharma et al.[21] reported hepatomegaly (58.5%), as the next most dominant clinical feature. However, a number of workers, have reported headache as the second most common symptom after fever (75.34-94.75%). Generally, these clinical features are generalized and are non-specific in nature. In present study too, we found that most of these features except for hepatomegaly had no differential value in differentiating cases with bleeding manifestations from those not having bleeding manifestations.

In present study, mean hemoglobin value was observed to be 12.43±2.56 g/dl with 16% patients having haemoglobin levels <10 g/dl, thus showing that haemoglobin levels are less affected in patients of dengue fever. In present study and number of other studies no significant difference in haemoglobin levels of patients with and without bleeding manifestations was seen.

An overview of different studies showing these abnormalities in dengue fever patients is shown in [Table D1] below:

Table D1: Comparative evaluation of different hematological abnormalities in dengue fever in present study as compared to different contemporary series.

Author (Year)	Leucopenia (TLC<4000)	Raised Hematocrit	Platelet count <50,000
Vanamali et al. (2013) [13]	47%	-	30% (<40,000)
Hasan et al. (2013)[24]	34.12%	-	31.11%
Deshwal et al (2015)[26]	20.19%	-	69.51%
Unnikrishnan et al. (2015)[19]	52.8%	-	-
Patel et al. (2016)[22]	56.92%	11.54% (Cut-off>45%)	31.16%
Khan et al. (2016)[15]	38.66%	23.33% (Cut-off>45%)	56.7%
Nagaram et al . (2017)[20]	55.17%	26.44% (Cut-off >40%)	53.4%
Sharma et al. (2017)[21]	36%	55% (Cut-off >36.3%)	19%
Present study (2017)	24%	71% (Cut-off>35%)	47%

Hematological abnormality profile in present study was similar to that observed in different contemporary studies with slight differences owing to difference in profile of patients, environment and time of assessment.

As far as complications like bleeding manifestations were concerned, we found that among different hematological parameters only platelet count had a significant association with bleeding manifestations. These observations are in agreement with the observations of Vanamali et al and Kauser et al.[13,14] An association of platelet count with severity of dengue fever was also reported by Nagaram et al.[20], who reported that 46.3% of non-severe and 96% of severe cases in their study had platelet count <50,000/cumm. Even higher discriminant role of platelet count in assessment of severity of dengue fever was reported by Sharma et al.[21] who found that 10.7% of non-severe and 82.6% of severe DF cases had platelet count <50,000. In present study too, 34.5% of patients without bleeding manifestations and 62.2% of those having bleeding manifestations had platelet count <50,000. These findings thus endorse that not only the platelet count affected during dengue fever but it also correlates well with the clinical spectrum and possible outcome of patient.

In present study, liver functions (AST and ALT) and serum albumin levels were found to be heavily deranged in 77%, 80% and 57% patients respectively. Derangement of S. alkaline phosphatase levels was also seen but in relatively much lesser proportion of study population (8% only). Liver functions are heavily affected during dengue fever. In previous studies to the impact of dengue fever on biochemical levels has widely been studied and reported. In their study, Karoli et al.[17] reported elevated liver enzymes in as many as 92% of cases and hypoproteinemia in 34% of cases while Deshwal et al.[26] (2015) reported elevated AST/ALT in 88.54% cases. Hasan et al.[24] reported the derangement of ALT in 42.2% cases. Unnikrishnan et al.[19] in their study among elderly patients with DF reported elevated liver enzymes in as high as 92.5% cases. Conversely, Sharma et al.[21] in their study among children with DF reported elevated liver enzymes in 12.5% and 22.5% cases only. However, Nagaram et al.[20] in their study among children reported deranged liver enzymes in as high as 87.36% patients. These findings in turn suggest that liver functions in general are affected in dengue fever, however, extent of their derangement might vary from study to study depending upon the study characteristics.

The present study witnessed, declining trends of platelet till day 3 of admission and thereafter showed a recovery trend with mean platelet count being 75±72, 71±53 and 92±54 thousands/cumm respectively. Thus present study witnessed minimum platelet count on day 3 of admission. Compared to this Lin et al.[27] reported the minimum count on day 5th-7th after fever onset. However, the present study differs from the said study as in present study evaluation was done on 3rd and 5th day after admission and not after the onset of fever. Azin et al.[16] in their study reported that laboratory abnormalities generally start on the 3rd day but are more evident on the 5th day with restoration of values by the 11th day. Vanamali et al.[13] also reported the minimum platelet count on day 5 of illness. In present study, the difference in pattern of changes in thrombocytopenia could be owing to the fact that the present study was carried out at a tertiary care centre where most of the cases of DF were referred from primary and secondary care services and hence, the chronology of changes in laboratory parameters could vary slightly keeping in view the fact that we made assessments from the day of admission rather than from the day of onset of illness. However, the present study endorses the recovery trends in platelet count as reported by Jameel et al.[28] who reported platelet count of 55000 and 85000/cumm respectively on day 1 and day 5, thus showing that on day 5 the recovery trend of platelets is initiated among patients undergoing treatment.

CONCLUSION

The findings of present study showed that hematological and biochemical profile of dengue fever is deranged substantially. The clinical profile of patients with more severe dengue fever (bleeding manifestations in present study) was marked by higher proportion of hepatomegaly and lower platelet count. The clinical course of disease is marked by a rationalization of platelet count by day 5 of illness. The findings in present study provide a deep insight into the hematological picture vis-à-vis the clinical and biochemical profile of dengue fever at a tertiary care centre located in a region dominated by rural and suburban patients who are often referred by primary and secondary care providers in view of the complicating and serious stage of illness. Considering the relevance of onset and hematological changes, the findings in present study were somewhat skewed as most of the patients included in the present study had delayed admission and as such the onset record was not available. The present study was also limited by the duration of follow-up and outcome evaluation, including relapse. Hence, further studies with longer duration of follow-up and outcome evaluation, and focus on calculating the time delay between onset of fever and admission to our facility might provide some valuable clue that might help in understanding this relationship further.

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