Original Article

Study of Haematological Profile of Dengue Fever and its Clinical Implication

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ABSTRACT

Background: Dengue virus, a mosquito-borne human viral pathogen, has recently become a major national health problem.The disease is transmitted in rainy season. There are four dengue virus serotypes, called DEN-1, DEN-2, DEN-3, and DEN-4. The clinical manifestation of dengue infection varies from asymptomatic to severe life threatening illness in the form of DHF/DSS. Dengue haemorrhagic fever or DSS may be fatal in 40% to 50% of untreated patients. Laboratory diagnosis of dengue virus infection depends upon demonstration of specific antibodies in serum samples. Early diagnosis and monitoring is largely dependent on haematological parameters. As no specific antiviral therapy is available, supportive therapy is of most importance.

Methods: NS1, Ig M & Ig G detection by Dengue Day 1 Test kit, haematological parameters by automated analyzer & peripheral smear, coagulation profile analysis by ACL-7000 coagulation meter, LFT by BACKMAN COULTER AU480.

Result: Out of 100 cases, 95 (95%) of the patients had fever, 39 (39%) cases had leukocyte count <4,000/ mm3, thrombocytopenia (<1,00,000/mm3) was observed in 81, 48 cases had derranged LFT with thrombocytopenia,17 cases had prolonged APTT which correlated with thrombocytopenia, 14 cases had raised APTT & deranged LFT which also showed thrombocytopenia.

Conclusion: Thrombocytopenia was most predominant haematological discrepancy. Most cases (70%) were of classical DF & (28%) were cases of DHF who had fever, showed thrombocytopenia & deranged haematological parameters which signifies disease severity. 2% cases presented with hypotension & altered consciousness with severe thrombocytopenia & prolonged APTT (DHF/DSS) which indicates very poor prognosis & had fatal outcome. Initial leukopenia & leukocyte count returning to normal by ninth to tenth day after therapy in most of the cases indicates that leukocyte count an important benchmark for clinical improvement. Dengue fever does not have specific medical therapy hence clinical recovery monitoring is largely dependent on haematological parameters.

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Introduction

Dengue fever is a viral infection transmitted by Aedes aegypti mosquitoes. Dengue is now endemic in over 100 countries with dramatic increase in geographical range recorded in recent years[1].

Humans are the main amplifying host of the virus. Rainy season or post rain season favours the collection of water in various sites like old tyres, coolers, old earthenware pots, coconut shells etc. This acts as a good site for mosquito breeding[2]. Navi Mumbai is an endemic area for dengue. Dengue virus infections results in wide spectrum of clinical manifestations ranging from asymptomatic to symptomatic disease as classical dengue fever, dengue haemorrhagic fever with and without shock syndrome, and unusual manifestations including encephalitis, liver failure and severe bleeding without shock.

Clinical presentations depend on age; virus strain and immune status of the host[3].Dengue virus cause 2 forms of clinical syndromes.

(1) Classical dengue fever (DF) - The onset is sudden with chills and high fever, intense headache, muscle pain, joint or bony pain (Break bone fever), retro orbital pain and photophobia. Other common symptoms include weakness, abdominal pain, sore throat and general depression. Some patients with dengue fever have evidence of mucosal or cutaneous bleeding without other evidence of DHF/DSS like haemoconcentration or fluid leak; such patients are classified as dengue fever with unusual bleeding[4] .It may be associated with leukopenia and thrombocytopenia[5].

(2) Dengue haemorrhagic fever/Dengue shock syndrome (DHF/DSS) - After an incubation period of four to six days the patient develops clinical features like of dengue fever. There may be varying degree of tender hepatomegaly or less commonly splenomegaly. All patients have some degree of haemorrhagic phenomenon like positive tourniquet test, petechial spots, bruising at venepuncture site, bleeding from gums, epistaxis, hemetemesis or melena, muscle hematoma, hematuria and rarely intracranial haemorrhage may occur. DHF/DSS shows thrombocytopenia (platelet count <1,00,000/cu.mm) and >20% of rise in average haematocrit[6].

The most significant pathophysiologic changes among dengue virus infections are seen in DHF/DSS, due to plasma leakage from intravascular to extravascular compartments. The leakage of plasma leads to hemoconcentration, hypotension, hypoproteinemia and collection of fluid in serous cavities. The plasma leakage occurs as a result of acute increase in vascular permeability which is attributed to transient functional disturbance due to action of short acting chemical mediators. Immune reactions contribute to the formation of complexes that initiate intravascular coagulation or haemorrhagic lesions. Damage to circulatory vessels appears to occur when antigen antibody complexes activate the complement system with the release of cytokines[7].All these inflammatory response produces vasculopathy and also increases shock[8].

Laboratory diagnosis of dengue virus infection depends upon demonstration of specific antibodies in serum samples by haemagglutination inhibition, complement fixation, neutralization test or ELISA. Reverse transcriptase PCR and hybridization probes for nucleic acid are other newer tests for diagnosis. Hence this study was undertaken to predict prognosis of dengue infection with help of haematological parameters.

As there is no specific antiviral treatment, management is essentially supportive and symptomatic. Early and effective replacement of losses with plasma, plasma expander and/ or fluid and electrolyte solution results in a favourable outcome in most cases.

Materials and Methods

The study includes clinically suspected 100 cases of dengue with serological confirmation of either dengue specific NS1 antigen assay and/or IgM and/or IgG antibodies detection were selected. Evaluation of hematological and coagulative parameters and peripheral smear study were carried out. Serologically positive cases of dengue which were also positive for other coexistent infections E.g. Malaria, typhoid, are excluded from the study. Paediatric age group was excluded.

Serology derived from a venous blood sample was collected from the patients presenting with symptoms suggestive of dengue. A commercially available Dengue Day1 test kit was used to detect NS1 antigen and IgM and IgG antibodies. The test results were expressed as positives/negatives for antigen and both antibodies.

Evaluation of hematological parameter was done by collecting 2 ml of samples on EDTA prefilled bottles which were examined for Haemoglobin count, Haematocrit, Platelet count, Total leucocyte count, Differential leucocyte count. The analysis is done by the automated analyzer SYSMEX XT 1800i(3-part differential) /ADVIA 2120i (5-part differential). APTT/PT was done by collecting 2ml blood on sodium citrate prefilled bottles. The analysis was done by ACL-7000 coagulation meter. Evaluation of LFT was done by BACKMAN COULTER AU480.

Result:

One hundred patients, diagnosed as dengue based on NS1, Ig M & Ig G positivity & haematological parameters with LFT & coagulation profile were done.

In our study out of 100 patients, 70 (70%)cases were males and 30 (30%)cases were females. The male : female ratio

was 2:1. Out of 100 patients, 49 (49%) were in age group of 15 to 25 years followed by 33 (33%)cases in the age group of 26 to 35 years, 10 (10%)cases in the age group of 36 to 45 years and 5 (5%)cases with age more than 55 years,3 (3%) cases were recorded in the age group of 46 to 55 years. In this study, 95 (95%) of the patients had fever as presenting symptom. Other symptoms like myalgia in 70 (70%)cases, arthralgia in 60(60%)cases, headache in 50 (50%)cases.

Table 1: Rise In Haematocrit

HCT (%)	No. of Patients	Percentage
>40%	28	28%
<40%	72	72%

Table 2: Distribution of Patients According to Leukocyte Count (/mm^3)

Leucocyte Count (/mm^3)	No. of patients	Percentage
<4,000	39	39%
4,000-11,000	49	49%
>11,000	12	12%

Table 3: Distribution of Patients According to Platelet Count (/mm^3)

Platelet count (/mm^3)	No. of patients	Percentage
<20,000	10	10%
20,000-50,000	37	37%
50,000-1 lakh	34	34%
>1 lakhs	19	19%

Table 4: Distribution Of Patients According To APTT (/sec) (Out of 63 cases)

APTT (/sec)	No. of patients	Percentage
Normal	46	73%
Raised	17	27%

Table 5: Distribution Of Patients According To Liver Function test(LFT) (Out of 76 cases)

LFT(AST &ALT)	No. of patients	Percentage
Normal	33	43%
Increased	43	57%

Table 6: Correlation Between Haematological Finding & LFT (Out of 76 cases)

	Platelet Normal	Platelet Decreased
LFT Increased	No cases	48
LFT Normal	1 case	28

Table 7: Correlation Between Haematological Finding & APTT (out of 63 cases)

	Platelet Normal	Platelet Decreased
APTT Increased	No cases	17
APTT Normal	No cases	46

Table 8: Correlation Between LFT & APTT

	LFT Increased	LFT Normal
APTT Increased	14	3
APTT Normal	18	48

Discussion

Haematological profile of 100 cases were done which were confirmed by serologically.

Study include ,81 cases were NS1 positive, 16 cases were Ig M positive & 5 cases showed positivity for both.15 cases were IgG positive. Primary dengue virus infection is characterized by elevations in specific NS1 antigen levels 0 to 9 days after the onset of symptoms; this generally persists upto 15 days. Ig M positivity indicates recent primary infection & IgG positivity indicates recent secondary infection.

Out of 100 patients, majority 82(82%)cases were in age group of 15 to 35 years,13 (13%) patients were in age group 35 to 55 years and 5(5%) were more than 55 years of age**.** Most of these patients were adults, this may be due to the fact that most of them are working population, construction site labourers & travellers. Habitats for Aedes aegypti are domestic containers, stagnant water, ornamental containers and roof gutters.These findings were comparable with a study conducted by Fu Xi Qiu et al[9] in which 81% of the patients were among more than 20 years. 70 (70%) patients were male and 30 (30%) were females with a male to female ratio of 2:1**.**This is due to the fact that males predominantly form the working population & more prone to infection by mosquito bite in a day time. These findings were comparable with a study conducted by Agarwal et al[10] in which male to female ratio was 1.9:1.

In our study, haematocrit was raised (> 40%) in 28 (28%) cases**.** Study done by Nazish Butt et al[11] showed raised haematocrit (>40%) were found in 52(50%) of patients.It is higher than our finding of 28%. This is probably due to the fact that they had more cases of DHF/DSS.

In DHF and DSS, there is an acute increase in vascular permeability that leads to leakage of plasma into the extra-vascular compartments, resulting in haemoconcentration and hypotension.[12]

In our study, thrombocytopenia (<1,00,000/mm^3) was observed in 81% of the patients.Among them 10 (10%) patients had severe thrombocytopenia (<20,000/mm^3), 37(37%) patients had moderate thrombocytopenia (20,000 to 50,000/mm^3), while 34(34%) patients had mild thrombocytopenia (50,000 to 1,00,000/mm^3).)Only 19(19%) patients had platelet count >1,00,000/mm^3.

A study done by Narayanan et al [13] in 59 patients, 14 patients had a platelet count less than 50,000cells/cu.mm ;29 patients had platelet count between 50,000-1,00,000 and only 16 patients had platelet count more than one lakh .

Cause of thrombocytopenia is controversial, but the possibilities include impaired megakaryocyte production earlier in the disease, platelet injury by virus itself, platelet specific antibodies, immune complexes or DIC.

Out of 100 cases, 28 (28%) patients had bleeding manifestations who had platelet count less than 30,000/ cu.mm & these cases were clinically diagnosed as DHF.70(70%) cases were classical DF & 2 cases had evidence of circulatory failure. DHF is due to antibody mediated immune enhancement which causes platelet destruction. Shock in DSS occur due to sudden extravasation of plasma to extravascular sites like the pleural and abdominal cavity. This occurs because of increased vascular permeability due to immune activation.

In our study, 17(17%) cases had evidence of pleural effusion & 3(3%) cases had ascites. Both categories were associated with thrombocytopenia .

In our study out of 63 patients, 46(73%) had normal APTT , 17(27%) patients had raised APTT & 7 patients had raised PT. Raised APTT correlates with finding of severe thrombocytopenia. Out of these 2 patients had clinical evidence of DSS**.**

However ,most patients(73%) had normal APTT . So, prolonged APTT was not a significant finding in our study. As opposed to a study conducted in Taiwan in 2003 which showed that 77 patients out 79 (97.5%) patients with DHF had prolonged APTT and 33 out of 48 (68.8%) had prolonged APTT in classical dengue fever patients[14]. In this study cases of DHF were more so prolonged APTT was significant. But in our study 70% cases showed classical dengue fever which were not associate with prolonged APTT. So this finding was not significant . Prolongation of APTT is due to excess activation of fibrinolysis during infection causes increased tendency of bleeding[15].This phenomenon occurs in cases of DSS. Leukopenia was more common in DF and DHF Grade I and II. DSS did not show leukopenia. Neutropenia may be due to marked degeneration of mature neutrophils and "shift to left" during febrile phases of illness.[13]However this parameter can be used as a benchmark for clinical improvement[15].

In our study, out of 100 patients 39 (39%) cases had leukocyte count <4,000/ cu.mm,49(49%)cases had leukocyte cases count 4,000 – 11,000/cu. mm and 12(12%) cases had leukocyte count >11,000 cu.mm. Leukopenia was more common in DF and DHF Grade I and II.DSS did not show leukopenia**.** Similar findings were found in study done by Krishnamurthy K. et al[16] in 1964, observed that out of 89 cases, 28 (31.46%) had leukopenia (<5,000/ mm3), remaining 61 (68.54%) cases had normal leukocyte count and there was no leukocytosis in any of the cases.

In our study, out of 100 cases 50(50%) cases had lymphocyosis which show presence of plasmacytoid lymphocytes**.**It has been suggested that the atypical lymphocytes in secondary dengue infection can be representative of augmented immune response in an attempt to control the spread of dengue virus-infected cells.[17]

Plasmacytoid lymphocytes have been reported by John Gawoski and Winnie Ooi which mainly represents that serum immunoglobulin production is enhanced during dengue viral infection which correlated with our study.[18]

In our study, out of 76 cases, 43(57%) cases had deranged LFT. These 43 cases correlated with haematological discrepancy**.**Our findings were compared with Fu-Xi Qi et al[9] study observed overall abnormal LFT in 68 (44.2%). This is due to virus is particularly found in the hepatocytes, kupffer cells and the endothelium, along with immune complex formation. It has been suggested that it may be due to excess release of AST from damaged myocytes during dengue infections.

In our study majority that is 95 (95%) of the patients had fever as presenting symptom. Other symptoms like myalgia in 70 (70%), arthralgia in 60(60%), headache in 50(50%), backache in 40(40%), abdominal pain 30(30%) ,petechia 40(40%),pleural effusion 17(17%),ascites 5(5%) and subconjuctival haemorrhage 3(3%). Altered sensorium due to intracranial bleed or hypoxia of brain due to hypotension was present in 2 (2%) case**.**

All cases(95%) of fever correlated with thrombocytopenia. 5(5%) cases presented with weakness & bodyache which showed a normal platelet count. DSS(2%) patients presented with altered sensorium & hypotension had fatal outcome.

A study conducted by Sharma et al[19] showed that most commonest presenting symptom among the dengue cases

was fever (100%). The symptoms reported included bodyache (98%), vomiting (28%) loose motion (12.7%), abdominal pain (10.5%), skin rash (43.1%) and altered sensorium (0.5%).

In our study ,we received post therapy samples of 51 cases. Out of these 2 cases were fatal & did not show any recovery & had bad prognosis.49 cases showed improvement in platelet count within 5 to 6 days after therapy. In these patient platelet counts were improved from 40,000 /cu.mm to 2 lakh after therapy & had good prognosis.

Conclusion

In our study 100 serologically confirmed cases of dengue viral infection were studied to analyze the haematological parameters which will help in predicting prognosis of disease.

Most cases (70%) were of classical DF & (28%) were cases of DHF who had fever, showed thrombocytopenia & deranged haematological parameters which signifies disease severity.

2% cases presented with hypotension & altered consciousness with severe thrombocytopenia & prolonged APTT (DHF/DSS) which indicates very poor prognosis& had fatal outcome..

Thrombocytopenia was most predominant haematological discrepancy. Raised haematocrit was not significant significant (72% had normal haematocrit) in our study & correlated with uncomplicated DF cases. Cases with raised LFT (57%) correlated with thrombocytopenia. APTT prolongation was significant in cases of DHF & DSS but not significant in cases of dengue fever. Pleural effusion was found in 17% of cases who had severe thrombocytopenia.

Leukopenia was present in 39% of cases. Initial leukopenia &leukocyte count returning to normal by ninth to tenth day after therapy in most of the cases indicates that leukocyte count an important benchmark for clinical improvement.

Cases with lymphocytosis (50%) with atypical lymphocytes were representative of augmented immune response in an attempt to control the spread of dengue virus-infected cells.

Conclusion

Dengue fever does not have specific medical therapy hence clinical recovery monitoring is largely dependent on haematological parameters.This study concludes that parameter like platelet count, haematocrit, leukocyte count & coagulation studies aid greatly in clinical monitoring of patients.

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Competing Interests

None declared

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