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Changing Epidemiology of Dengue Fever: Newer Insights and Current Concepts

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Authors' contributions

This work was carried out in collaboration between all authors. Author AA is the primary consultant internist and contributed to the conception, design, draft, analysis, revision and final approval of the work to be published. Author VG was involved with data acquisition, statistical analysis and tabulation of data. Author MA contributed to revision of the manuscript, grammar and literature search for the study. All authors read and approved the final manuscript.

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ABSTRACT

Background: Dengue fever (DF) is the most common of the arboviral infections in humans and more than two-fifths of the world's population lives in areas potentially at risk for dengue. Effective vector control, prompt case detection and appropriate clinical management can reduce the mortality from severe dengue. The objective of this paper is to present current epidemiology of dengue in this part of the country and, additionally, reflect on some important clinical issues involved in the management of these cases. The global incidence of DF and dengue hemorrhagic fever (DHF) has increased dramatically in recent decades. Endothelium is the target of the immune-pathological mechanism in DF and DHF, its hallmark being vascular permeability and coagulation disorders. Plasma leakage or capillary leak syndrome (CLS) is the most specific and life-threatening feature of DHF and is considered to be the primary lesion that underlies DHF.

Materials and Methods: The present study is a four year (January 2013- December 2016), retrospective observational study. The study population consists of 264 male or female indoor patients with confirmed diagnosis of dengue fever. Inclusion criteria were discharge diagnosis with

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International Classification of Disease (ICD) code A90 - A91 and availability of minimal dataset (name, registration number, age, sex, diagnosis, symptoms, laboratory investigations, transfusions if any, co-infection, other co-morbidities, date of admission, date of discharge, and discharge status). Indoor files of all these 264 cases were collected from medical records department and data tabulated. The study focuses on incidence and outcomes of CLS in dengue infection along with other various issues among these patients.

Results: In all 264 patients were included in the study with a male female ratio of 1.6:1. The youngest and the eldest patient were of 13 year and 95 year age respectively. Primary dengue fever (pDF) was seen in 219 (83%) and secondary dengue fever (sDF) in 45 (17%) cases respectively. Besides fever, chills, vomiting, abdominal pain, body-ache, bleeding manifestations, and hepato-splenomegaly, features of CLS were present in 164 (62.1%) cases. None of the cases had only left side pleural or pericardial effusion. In all 258 (97.7%) cases had thrombocytopenia, 39 (14.8%) cases had bleeding manidestations, 11 (4%) cases had severe organ involvement, 19 (7.2%) cases had co-infections, 43 (16.3%) cases required medical intensive care unit (MICU) admissions, and there were only 4 (1.5%) mortalities. The pathophysiology and management challenges of CLS along with other parameters are discussed.

Conclusion: Currently, no specific antiviral therapy exists for dengue virus. With prompt case detection and appropriate clinical management, the case fatality rate (CFR) is declining and the overall CFR in the southeast Asia region is now less than 1%. In our case series it was 1.5%. We conclude that patients of DF with thrombocytopenia and transaminitis should be evaluated early by ultrasonography for CLS. Hemoconcentration and hypoproteinemia are not sensitive tools to pick up capillary leakage in clinical practice. Isolated left side pleural effusion and pericardial effusion are not seen in DF and if present one should look for another cause. The case series raises question on the accepted theory of relation between capillary leakage and severe dengue infection. Further, since capillary leakage was seen in 78.8% of primary dengue infection cases, it also questions the validity of the immune enhancement theory of DHF/severe dengue. Viral burden may be a key factor in determination of disease severity rather than sequential infections or secondary infection. Early detection and appropriate case management practices are critical factors for survival.

Keywords: Dengue fever; primary dengue fever; secondary dengue fever; dengue hemorrhagic fever; dengue shock syndrome; capillary leak syndrome.

1. INTRODUCTION

The burden and the potential threat to global health by DF has been recognized globally and it is the most important mosquito-borne viral disease with 2.5 billion people at risk worldwide. Transmission of dengue is now present in almost every World Health Organization (WHO) region of the world and more than 125 countries are known to be dengue endemic. The epidemiology of dengue involves changes in the human host, the dengue virus and also the vector bionomics. Several studies have reported a shift in affected age groups, sex differences, expansion to rural areas, and virulence and genotype of the virus. The Aedes mosquito has evolved in longevity and survival, is affected by seasonality and climate variability, and also socio-cultural and economic factors of human habitation and development.

The typical clinical manifestations of dengue infection range from self-limited DF to DHF with shock syndrome. The pathogenesis is complex with the involvement of different immune factors. After the incubation period which ranges from 3-14 days, the illness begins abruptly and in patients with moderate to severe disease, is followed by three phases- febrile, critical and recovery. During the transition from the febrile to afebrile phase which lasts for 2-7 days, patients without an increase in capillary permeability improves without going through the critical phase. On the other hand patients with increased capillary permeability show warning signs due to capillary leakage. This period of clinically significant plasma leakage usually lasts for one to two days. The degree of plasma leakage varies from patient to patient with severe plasma leakage leading to shock (dengue shock) and/or fluid accumulation with respiratory distress. It is considered as the marker of severity for severe dengue.

The expansion of dengue is expected to increase in future due to factors such as the

epidemiological changes, rural expansion, seasonality and climate variability, sociocultural and socioeconomic factors, vector longevity and survival, globalization, travel, trade, and also viral evolution. Effective vector control is the mainstay of dengue prevention and control. Surveillance and improved reporting of dengue cases is essential to measure the true global situation as indicated in the objectives of the WHO Global Strategy for Dengue Prevention and Control, 2012–2020. More data will help in prioritization of research, formation of health policies, and allocation of financial resources toward reducing this poorly controlled disease. We present and discuss the findings in 264 cases of DF seen by us during 2013 to 2016 in our institution in Jaipur (Rajasthan, India). The objective of this paper is to review the current epidemiology of dengue infection in this part of the country, discuss about the features and severity of primary versus secondary dengue infection and, additionally, look into the prevalence and challenges to prevailing views of CLS in dengue infection. It does not focuses on societal factors, and vector or vaccine issues.

2. MATERIALS AND METHODS

The present study is a four year retrospective observational study comprising of 264 indoor patients of confirmed DF and was conducted in the department of internal medicine, Narayana Multispeciality Hospital (a tertiary medical care center), Jaipur (Rajasthan-India). Our institutional ethics committee approved this retrospective study and informed consent was not required. Medical case record files of all admitted cases of DF, in medical unit 1, from January 2013 to December 2016 were collected from medical records department (MRD). Demographic data, symptomatology, co-morbidities, co-infections, complications, dengue NS1 antigen, IgM / IgG test reports (done by dengue solid phase immune-chromatographic rapid test kits for qualitative detection), routine laboratory investigations, ultrasonography, x-ray chest, in addition to blood and blood product transfusions and mortality data were collected, tabulated and analyzed. Other viral fevers, other arthropod-borne fevers, unspecified viral fever, other viral or unspecified hemorrhagic fevers and bacterial diseases including leptospirosis were not included in the study.

Laboratory diagnosis methods for confirming dengue virus infection may involve detection of the virus, viral nucleic acid, antigens or antibodies, or a combination of these techniques. During the early stages of the disease, virus isolation, nucleic acid or antigen detection can be used to diagnose the infection. At the end of the acute phase of infection, serology is the method of choice for diagnosis. In this study dengue NS1 antigen and antibodies (IgM / IgG) test reports (done by dengue solid phase immune-chromatographic rapid test kits for qualitative detection) were evaluated. Subjects with positive NS1 antigen, or IgM antibodies or both were diagnosed to have primary DF. Subjects with positive NS1 antigen or IgM antibodies along with IgG antibodies or presence of all three was considered to have secondary DF (Table 2).

All these patients were routinely followed –up for capillary leakage by Thoraco-abdominal ultrasonography and other complications by physical examination, laboratory tests, x-ray and a chemistry panel on admission and repeated as needed afterwards. Thoraco-abdominal ultrasound is a highly accurate method for assessing even small amounts pleural effusion/ascites, and has a sensitivity of nearly 100%. It is even recommended in the WHO guidelines as a suitable tool for assessing capillary leakage in dengue patients. Sonographic evidence of capillary leakage was considered to be present if either of ascites, pleural effusion, or a thickened, edematous gallbladder were present. Gallbladder (GB) wall thickening was measured by placing the callipers between the two layers of the anterior wall. Subjects who had GB wall thickness >3 mm as measured on ultrasound were identified as positive for GB wall edema. Thoracic scanning was done in either sitting or supine posture. Both the pleural spaces were evaluated through an intercostal approach. Liver measuring more than 15 cm was taken as hepatomegaly and spleen measuring more than 12 cm was taken as splenomegaly. The paper reviews the changing epidemiology and symptomatology of DF.

3. RESULTS

In all 264 cases of confirmed DF case records were screened and analyzed. There were 163 (61.7%) males and 101 (38.2%) females. The median age was 25.5 years in males and 31 years in females, and the male-female ratio was 1.6:1 (Table 1). The youngest and the eldest patient were of 13 years and 95 years respectively.

Table 1. Distribution of 264 cases with age group

S. no.	Age category	No. of male cases	No. of female cases	Total	Percentage
1	0-10	0	0	0	0
2	11 to 20	38	22	60	22.7
3	21-30	70	27	97	36.7
4	31-40	26	26	52	19.7
5	41-50	17	10	27	10.2
6	51-60	9	9	18	6.8
7	61-70	2	4	6	2.3
8	71-80	1	1	2	0.7
9	81-90	0	1	1	0.4
10	91-100	0	1	1	0.4
	Total	163	101	264	

The result of serology tests for dengue fever (NS1, IgM, IgG antibody) are shown in Table 2. pDF was seen in 219(83.0%) and sDFin 45(17.0%) cases. The clinical manifestations are mentioned in Table 3. Fever was present at admission in almost all cases (97.3%), followed by chills (56.8%), vomiting (54.5%), hepatomegaly (47%), abdominal pain (46.2%), and bodyaches 100(37.9%). Bleeding manifestations were seen in 39(14.8%) cases. Other symptoms were nausea (37.5%), splenomegaly (25%), weakness (21.6%), and loss of apetite (19.3%). Diarrhoea, rash, itching and cough were not very common, being seen in <15% of patients. Headaches and retro-orbital pain described as classical symptom were seen in only 21.2% cases.

Table 2. Serological markers in dengue cases

Antigen/ antibody detected	Number of cases		Total
	Male	Female	
NS1 only	128	72	200
IgM only	4	3	7
NS1+IgM	9	3	12
NS1+IgG	8	5	13
IgG and IgM	6	7	13
NS1, IgM, IgG	8	11	19
Total	163	101	264

Hematocrit more than 37.5% was seen in 159(60.2%) cases (Table 4). Only 14(5.3%) cases had a hematocrit of >50%. Hypoalbuminemia was seen in 103 (39%) cases (Table 4). It was moderate in 57(22%) and severe in 16(6%) cases. Transaminitis i.e., raised serum glutamic-oxaloacetic transaminase (AST), serum glutamic-pyruvic transaminase (ALT) and gamma glutamyl transpeptidase (GGT) were seen in 238(90.1%), 126(47.7%) and 65(24.6%) patients respectively (Table 5).

Table 3. Distribution of clinical manifestation

Clinical manifestations	No. of cases (%)
Fever	257(97.3)
Chill	150(56.8)
Vomiting	144(54.5)
Hepatomegaly	124(47)
Abdominal Pain	122(46.2)
Body-ache	100(37.9)
Nausea	99(37.5)
Splenomegaly	66(25)
Weakness	57(1.6)
Headache/Retro orbital pain	56(21.2)
Loss of Appetite	51(19.3)
Bleeding	39(14.8)
Diarrhea	30(11.4)
Rashes	29(11)
Cough	23(8.7)
Itching	23(8.7)

Thrombocytopenia was present in 258(97.7%) cases (Table 7). Mild thrombocytopenia was present in 14(5.3%), moderate in 33(12.5%) and severe in 211(79.9%) cases. It was below 20 x 10^3/cmm in 125(42.3%) cases. 38(14.4) cases had platelet counts $\leq$ 10x10^3/cmm. Bleeding manifestation (Table 7) were present in 39 (14.8%) cases; melena (13 patients) being the commonest, followed by menorrhagia, epistaxis, hematuria, hemoptysis, petechiae, gingival bleed and surgical site bleeding. All these patients received platelet transfusion except one. Lowest platelet count was 3x10^3/cmm. One patient with platelet count 77x10^3/cmm had severe life threatening post partum hemorrhage. Severe critical thrombocytopenia (<20 x 10^3/cmm) was seen in more of primary dengue fever cases as compared to secondary dengue cases(49.8% v/s 35.5%).

Dengue with warning signs such as fluid accumulation were seen in 164(62.1%),

vomiting 144(54.5%), Hepatomegaly 124(47%), abdominal pain 122 (46.2%) and mucosal bleeding in 39(15.2%) cases respectively. Features of Severe dengue such as severe plasma leakage leading to shock (dengue shock syndrome) or fluid accumulation with respiratory distress were seen in 61(23.1%), severe bleeding (as evaluated by a clinician) requiring transfusion in 29(11%) and severe organ involvement (i.e., AST or ALT 1000 IU/L or greater, impaired consciousness, organ failure) in 11(4%) cases. 43(16.3%) required MICU admission.

Our results indicate that CLS is not uncommon in dengue fever, being present in 164 (62.1%) cases. Site of plasma leakage is mentioned in Table 8. GB wall edema was commonest followed by ascites and pleural effusion. Polyserositis with ascites,pleural effusions and GB wall edema were seen in 76((28.8%) patients. Interesting finding was that none of them had isolated left side pleural effusion or pericardial effusion.

Incidence of co-infection, co-morbidity, MICU admissions and mortality is shown in Table 9. Co-infection was seen in 19(7.2%) patients. Scrub typhus was seen in 10(3.8%), plasmodium vivax malaria in 4 (1.5%), and falciparum malaria in 4(1.5%) patients respectively. 1 (0.4%) patient had co-infection with plasmodium falciparum as well as plasmodium vivax. Co-morbidities such as diabetes mellitus, hypertension, coronary artery disease, COPD, benign prostate hyperplasia requiring indwelling catherization were seen in 39(14.7%) cases. MICU admission were required in 43(16.28%) cases.

In all there were 4(1.5%) mortalities. All of them had primary dengue fever (NS1 antigen positive). First one was an elderly 70 years male ,post CABG and reported to triage on 37th post operative day with fever and bleeding from surgical site. His lowest platelet count were 29x10^3/cmm. He had hypotension and evidence of organ dysfunction such as adult respiratory distress syndrome, acute kidney injury, severe transaminitis (AST 8400 IU/L and ALT 5329 IU/L) and features of CLS. Second patient was a young 30 years male who presented with altered sensorium and had largeintra cerebral bleed, hemoptysis, hemetemesis, melena ,transaminitis and lowest platelet counts of 34x10^3/cmm. He was declared brain dead. The third patient, 40 years female had acute pancreatitis, transaminitis, hemoconcentration with lowest platelet count of 11x10^3/cmm. She had sudden death after 10 hours of admission. Autopsy was declined by the family. The fourth patient, 25 years male had acute pancreatitis, severe transaminitis (AST 19450 IU/L, ALT 6838 IU/L), CLS, mild jaundice, melena, and lowest platelet count of 33x10^3/cmm. All four of them were given blood and blood products transfusion as needed.

Table 4. Hematocrit and serum albumin value in dengue cases

Test	Total no. of case (%)	CLS cases	Non CLS cases
Hematocrit (%)			
37.5-40	39(14.8)	22	17
40.1-50	106(40.1)	70	36
50.1-60	14(5.3)	11	03
Serum albumin (grams/dl)			
< 2.5	16(6.1)	13	03
2.5 – 3.0	57(21.6)	46	11
3.0 – 3.2	30(11.4)	16	14

Table 5. Hepatic enzymes in dengue cases

Test	2-5 time upper limit			>5 <10 time upper limit			>10 time upper limit		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
AST (15-37IU/L)	48	40	88	57	27	84	42	24	66
ALT (30-65 IU/L)	69	37	106	11	7	18	8	2	10
GGT (15-85 U/L)	40	17	57	6	2	8	0	0	0

AST: Serum glutamic-oxaloacetic transaminase; ALT: Serum glutamic-pyruvic transaminase; GGT: Gamma glutamyl transpeptidase (GGT)

Table 6. Thrombocytopenia in primary v/s secondary dengue

Pletlets count	Primary dengue cases (n=219)	Secondary dengue cases (n=45)	Total cases
>1.5 lac/cmm (Normal)	03	03	06(2.3%)
1 to 1.5 lac/cmm (Mild)	11	03	14(5.3%)
50 - 1x 10^3/cmm (Moderate)	27	06	33(12.5%)
20 – 50x 10^3/cmmk(Severe)	69	17	86(32.6%)
<20x10^3/cmm (critical)	109	16	125(47.3%)

Table 7. Bleeding manifestation in dengue cases with thrombocytopenia

S. no.	Pletelets count (cells/ cmm)	Menorrhagia	Epistaxis	Haemoptysis	Petechiae	Gum bleeding	Melena	Haematuria	Surgical site	Total patients with bleeding	Number of patients transfused
1	≤20x10^3	3	5	0	1	0	12	4	0	24	23
2	20 x10^3 to 50 x10^3	4	4	2	1	0	1	0	1	12	05
3	50 x10^3 to 100 x10^3	2	0	0	0	0	0	0	0	2	01
4	100 x10^3 to 150 x10^3	0	0	0	0	1	0	0	0	1	0

Table 8. Capillary leak syndrome in Dengue cases

Site of capillary leak	No. of cases (%) N=264
Left side pleural effusion	0(0)
Right side Pleural effusion	8(3.0)
B/L Pleural effusion	3(1.1)
Ascites	8(3.0)
GB wall edema	26(9.8)
Pericardial effusion	0(0)
Only Ascites + pleural effusion	9(3.4)
Only Ascites + GB wall edema	22(8.3)
Ascites + GB wall edema+ Pleural effusion	76(28.8)
Only Pleural effusion +GB wall edema	12(4.5)
Total CLS	164(62.1)

Table 9. Incidence of co-morbidity, co-infection, MICU admission and mortality in dengue cases (n=264)

	Male	Female	Total (%)
Co-morbidity	26	13	39(14.7)
Co-infection	09	10	19(7.2)
MICU admission	29	14	43(16.3)
Mortality	03	01	04(1.5)

Table 10 compares pDF cases with SDF cases. It was observed that cases with sDF45 (17%) were not more symptomatic than pDF 219(83%) cases. There was not much difference in the incidence of CLS, bleeding manifestations, or MICU admissions. However, cases with sDF had more severe transaminitis. All four mortalities seen in the case series were in pDF cases.

Table 10. Comparision of various indices in primary versus secondary dengue cases (n=264)

	Primary DF (%)	Secondary DF (%)
Total cases	219(83%)	45((17%)
CLS	133(75.2)	31(68.9)
Bleeding manifestations	31(14.2)	08(17.8)
SGOT >1000 IU/L	08(3.7)	03(6.7)
SGPT > 1000 IU/L	04(1.8)	03(6.7)
Mortality	04(1.8)	00
MICU	35(16)	08(17.8)

The average hospital stay duration was 4-6 days (Table 11). 23(8.7) cases stayed for more than a week.

Table 11. Duration of stay in hospital of dengue cases

Duration of stay in hospital in days	No. of case
1-3 days	87
4-6 days	154
7-9 days	20
10-12 days	02
13-15 days	01
Total patients	264

4. DISCUSSION

Dengue infection is caused by a group of four relatively strict human viruses and despite its high prevalence much of its pathophysiology is still unclear .It is postulated that half the world's population lives in countries endemic for dengue, underscoring the urgency to find solutions for dengue control. The consequence of simple DF is loss of workdays for communities dependent on wage labour. The consequence of severe illness is high morbidity and mortality rates, since tertiary level care required for DHF/DSS management is beyond the reach of most of the persons at risk. The data we have shared are from a tertiary care centre.

In South‑East Asian countries, where all the serotypes (DENV‑1-4) are circulating, DF had been typically considered a disease of early childhood aged 2-15 years. However, an evidence of increase of dengue incidence in older age groups, and shift in modal age has been reported in various studies from singapore, Indonesia, Bangladesh and Thailand [1,2,3]. In our study the majority of cases were in the age group of 11-40 years with maximum incidence in the age group of 21-30 years , 28 (10.6%) cases being more than 50 years of age. The eldest was a 95 years female and the youngest were 13 year male and a female patient. These data supports the observation of increasing age of DV infected patients. The trend for increased incidence among young adults has important implications for control and prevention. Whether these are real increases (based on population distributions), increases in the proportion of severe dengue/DHF/DSS (and, hence, the proportion hospitalised) but not DF, increased awareness or the result of improved classification and diagnosis needs further clarification.

There are many studies from South‑East Asia region that suggest higher ratio of males than females in DF/DHF hospitalized cases (India,

Bangladesh, Singapore and Malaysia), and only few studies suggest no difference in sexes [4,5,6].

However, almost all of these studies were hospital-based, thus, probably only represent those who access healthcare rather than the infected population [7].

Various studies have indicated the differences between sexes in term of severity of illness and case fatality ratio. Some studies have reported a higher rate of mortalities among females than males [8,9]. However in our series we found higher incidence of co-morbidity, co-infection, MICU admissions, and mortality among male patients. What could be an interesting finding in this series is a higher incidence of co-infection with scrub typhus in female patients (7 females versus 3 male patients). This could be due to social practices among rural areas and villages of females more often sleeping on ground rather than cots, working in fields and not using mosquito repellants. Whether it involves a simple societal/local factors or a different pathogenesis and an immune response is a matter of further research.

There are several reports of CLS in DF.Capillary leakage, the defining lesion of severe dengue is considered to be present if any of the following three features are present: a rise in the hematocrit equal to or greater than 20% above average for age, sex and population; a drop in the hematocrit following volume replacement treatment equal to or greater than 20% of baseline; signs of plasma leakage such as pleural effusion, ascites and hypoproteinemia [10,11]. Of these, hemoconcentration is usually diagnosed retrospectively, hypoalbuminemia is infrequent and clinical recognition of plasma leakage is a difficult sign. The hematocrit in CLS in DF is usually > 40%, but may be as high as 55-60%. The need to have area specific cut off values has been suggested and studies from Delhi and Chennai recommended such values [12,13]. We have taken a cut off 37.5% in our patients since we do not have our own area specific cut off [12]. This hemoconcentration is due to plasma leakage, beginning at the end of the febrile stage and continues for one to two days after defervescence of fever. Nevertheless, early diagnosis of CLS is essential to start volume replacement and indicates progression to DSS. In our case series a hematocrit of >40 was seen in 120 (45.4%) cases and when area specific cut off value (37.5%) is taken into consideration the number rises to 159(60.2%) as was done in a Chennai based study [12]. Hypoalbuminemia (< 3.2 gm/dl) was seen in 103(39%) cases suggesting that it is an infrequent finding. Thoraco -abdominal ultrasonography is a highly accurate method for assessing even small amounts of pleural effusion/ascites, and has a sensitivity of nearly 100% and is even recommended by the 2009 WHO guidelines as a suitable tool for assessing CLS [14,15,16]. Thus, instead of a rise in hematocrit and hypoalbuminemia, thoraco-abdominal ultrasonography is an important significant tool to pick up CLS early in adult. It picked up 164(62.1%) cases in this series.

Studies in India have demonstrated that among hospitalized patients with uncomplicated DF, the prevalence of sonographic capillary leak is high and ranges from 34% [15,16] to as high as 100 % [10]. It was 62.1% in our series of 264 DF cases. Ours being a tertiary centre some amount of selection bias may have been there as severe clinical cases of dengue are usually referred to a hospital. Nevertheless our results have reproduced a high prevalence of capillary leakage in patients with classical DF. VEGF, a potent permeability enhancing cytokine, is thought to play a pivotal role in mediating plasma leakage in DHF.

Of 264 cases of DF, 219(83%) had PDF and 45(17%) had SDF. CLS was found in 133(75.2%) and 31(68.9%) cases of pDF and SDF respectively. i.e., the incidence of CLS was almost same in primary as well as secondary DF cases. The mechanism that is considered to be the underlying cause of CLS is thought to be ADE (antibody dependent enhancement), i.e., immune enhancement of viral replication because of previous exposure to dengue virus as a cause of capillary leakage/severe disease. However, we question this theory as CLS is seen even with primary infection in significant number of patients. Probably CLS represent a fundamental mechanism of disease in dengue infection rather than a function of host immune status as reported by Meltzer and others [15]. Our results suggest that capillary leakage occur in most DF infection (three fifth in this case series) and uncommonly leads to clinically apparent phenomenon , severe disease with DHF or DSS and that it can be easily picked up by thoracoabdominal sonography. It may even be a universal finding at microscopic level [15]. Though we did not extensively study the issue of primary versus secondary infection, bleeding

manifestations, transaminitis and MICU admissions were also almost similar in secondary and primary dengue infection.

In CLS, collection of fluid frequently involves multiple sites. In our series, polyserositis was the commonest finding with ascites, pleural effusion and GB wall edema seen in 76(28.8%) cases. Isolated GB wall edema, right side pleural effusion, and ascites were seen in 26(9.8%), 8(3%) and 8(3%) cases respectively. None of the case had only left side pleural effusion or pericardial effusion .The similar findings were observed in others studies [17,18]. Why only isolated right pleural effusion is seen is probably an anatomical phenomenon. The right lung has more organ mass than the left lung and thus the amount of blood vessels is greater on the right side than the left side. Since capillary leak is the underlying mechanism, it tends to occur more on the right side. The same explanation can hold basis for absent pericardial effusion in DF associated CLS. Another issue is percutaneous drainage of the effusions. The fluid accumulation was rarely severe and none of our cases required aspiration. The fluid accumulation cleared within a weak in all cases and doesn't requires any specific treatment. In case, the fluid accumulation doesn't clears within a week, it should be investigated further for any other likely etiology.

Scrub typhus and malaria co infection with dengue fever has been reported in literature [19,20,21]. We had 19 (7.2%) cases with dual infection and all of them recovered. 10(4.3%) of them had co infection with scrub typhus. Malaria co infection was seen in 9(3.4%) cases. Four had plasmodium vivax, four plasmodium falciparum and one had mixed infection with plasmodium vivax and falciparum respectively. One of these case who had dual infection with dengue virus and plasmodium vivax malaria developed secondary hemophagocytic lymphohistiocytosis syndrome [22]. Persistent fever, transaminitis, presence of an eschar in one of them, and multi-organ involvement led us to suspect co-infection in them. A high index of clinical suspicion, early screening in suspected cases and failure of therapeutic response should raise an alarm to the possibility of co- infection.

Severe critical thrombocytopenia (<20x10^3/cmm) was seen in 125 cases (47.3%). Of them 109 had pDF and 16 had sDF. Only 24 of these 125 cases had some degree of bleeding manifestations and 23 received platelets transfusion. One of them with mild menorrhagia was not transfused as she showed rapid spontaneous recovery in platelet counts. These data suggest that a policy of restricted blood product transfusion should be practiced and only cases with evidence of significant bleeding at any platelet count be given therapeutic platelet transfusions. Majority of cases did not require platelets transfusion. Only 38 patients had platelet count $\leq$ 10 x 10^3/cmm and all were given prophylactic or therapeutic platelet transfusion. One patient with platelet count 77x10^3/cmm had severe life threatening post partum hemorrhage and was given recombinant factor VII [23].

Little is known about dengue in the elderly [24]. A surveillance study [25] showed that clinical manifestations of dengue in the elderly are similar to those of younger adults. In our case series we had 7 cases of DENF in elderly (>60 years age). Of them 5 cases had CLS and did not differ from other cases in their clinical manifestations and outcomes. As already discussed only one of them who had undergone CABG and had multiple co morbidities died following severe DHF.

The global case-fatality rate (CFR) for DHF/DSS has been declining in most of the endemic countries according to government statistics. The overall CFR in the southeast Asia region is now less than 1% [26]. However, disaggregated data reveal a different picture. Rates vary significantly between countries, provinces and hospitals, pointing to a more complex situation [27]. In our series CFR was 1.5% and could be due to the fact that ours is a tertiary referral centre with complicated cases being referred for management.

Secondary dengue infections or particularly virulent viral strains are two key factors thought to be associated with increased risk of severity [28,29]. In severe cases, thrombocytopenia and increased vascular permeability can result in hemorrhagic and shock complications. All four mortality in this case series were in primary dengue infection cases. We did not see secondary dengue infection being more severe than primary dengue infection. It appears that underlying pathogenesis is multifactorial with increased pathogenecity of specific serotype, virulence of the circulating virus, and immunological responses along with higher levels of viral loads and NS1 proteins implicated as critical for the appearance of DHF [30,31,32,33]. This suggests that viral burden

may be a salient factor in determination of disease severity rather than sequential infections or secondary infection. Genotyping or subtyping and viral load tests for DENV were not done in this study.

5. CONCLUSIONS

1. Early diagnosis, good supportive care, recognition and monitoring of fluid electrolyte status during the critical CLS phase are essential in the management of DF.
2. The modal age group affected by dengue has shifted from <15 years of age to 15-30 years of age. Even older age group are being significantly affected. We found higher incidence of co-morbidity, co-infection, MICU admissions, and mortality among male patients.
3. Thrombocytopenia and transaminitis are seen in all cases of CLS. This suggests that all patients of dengue infection with these two laboratory abnormalities should be screened early by ultrasonography for CLS and observed for DHF/DSS. Further, hemoconcentration and hypoproteinemia are not sensitive tools to pick up capillary leakage in clinical practice.
4. Isolated left pleural effusion and pericardial effusion are almost never seen in DF. If present, and in cases who fail to clear capillary leak in a week's time further evaluation should be done.
5. Capillary leakage is prevalent among patients with primary as well as secondary dengue infection. Probably CLS represent a fundamental mechanism of disease in dengue infection rather than a function of host immune status. Viral burden may be a key factor in determination of disease severity rather than sequential infections or secondary infection.
6. Co-infections are known with dengue fever. A high index of clinical suspicion, early screening in suspected cases and failure of therapeutic response should raise an alarm to the possibility of co- infection.
7. Since there is no specific treatment for DF, prevention through vector control and public health education is the key.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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