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VALIDATION OF SIGNIFICANT PROTEIN MARKERS FROM THE PLASMA OF SEVERE DENGUE INFECTED ADULT PATIENTS USING SELECTED REACTION MONITORING ASSAY

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ABSTRACT

Background: Infection with dengue virus (DENV) causes a spectrum of clinical manifestations ranging from mild dengue fever (DF) to the potentially lethal dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Dengue is endemic to the tropical and sub-tropical regions of the world, which are home to over half the population of the world as well as being popular tourist destinations

Aim : To study about information of the pathogenesis and clinical profile of dengue virus and Validation of significant protein markers of dengue infected adult patients using selected reaction monitoring assays.

Material &Methods : This study was conducted in Department of Medicine, Sardar Patel Medical College, Bikaner from July 2017 to December 2018. Total 26 cases with features of dengue illness along with positive dengue serology in this duration were admitted in hospital. This was a hospital based study.

Results : Proteomic analysis in severe dengue patients (DHF/DSS) shows down regulation of three significant proteins CD-44 antigen, complement component C8 beta chain and leucine-rich alpha-2-glycoprotein as compared to healthy controls. According to dengue agglutination test, 100% cases had IgM positive while 69.2% cases had NS1 positive. No case had IgG positive. Mean age in DHF group was 30.4812.58 and in DSS group mean age was 25.00 12.39 years and this difference was found statistically insignificant ($p>0.05$). Most common clinical manifestation was fever, abdominal pain and myalgia (100%) while rash was present in 96.2%, vomiting and headache were present in 92.3% cases each and least common clinical manifestation was retro-orbital pain (88.5%).Gum bleeding, epistaxis and petechiae were found in most cases.According to sensorium 96.2% cases were found normal and 3.8% cases were altered sensorium.According to outcome, 20 cases were cured successfully while 6 cases discharge on request. Patients were on telephonically follow up for one week, there was no mortality.

Conclusion : In present study revealed that validating 26 serum samples used for optimization by using selected reaction monitoring. Three significant proteins, CD44 Antigen, Complement component C8 beta chain, Leucine-rich alpha-2-glycoprotein are downregulated in severe dengue (DHF/DSS) patients as compared to healthy controls. These are predictive biomarkers of severe dengue fever (DHF/DSS).

KEYWORDS : DHF, DSS, Proteomics, DENV

INTRODUCTION

Infection with dengue virus (DENV) causes a spectrum of clinical manifestations ranging from mild dengue fever (DF) to the potentially lethal dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS)[1]. In humans, the major cellular targets of dengue appear to be dendritic cells of the skin, macrophages and monocytes[2].

Dengue fever was first reported in 1780, when Benjamin Rush described this condition as “Break bone fever” because it causes such severe aches in the joints. Dengue virus (DENV) is a mosquito-borne single-stranded RNA virus member of genus Flavivirus of the family Flaviviridae. It is mosquito borne viral infection with four serotypes, causing dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). It is estimated that worldwide more than 50 million infections occur each year including 500,000 hospitalizations for dengue hemorrhagic fever, mainly among patients with the case fatality rate exceeding 5% in some areas[3]. Dengue has been identified as one of the neglected tropical diseases by WHO as mentioned in their report on neglected tropical diseases (2010)[4]. Approximately 1.8 billion (more than 70%) of the population at risk for dengue worldwide live in Member States of the WHO South East Asia Region (SEAR) and Western Pacific Region, which bear nearly 75% of the global disease burden due to dengue[5].

MATERIAL AND METHODS

This study was conducted in Department of Medicine, Sardar Patel Medical College, Bikanerfrom July 2017 to December 2018. Total 26 cases with features of dengue illness along with positive dengue serology in this duration were admitted in hospital.

Type of the Study: Hospital based comparative cross sectional study.

Selection criteria for dengue illness

Inclusion Criteria

- Adults≥18 years who were serologically positive with dengue illness were included.
- Malaria infection was rule out by microscopy examination and RDT.
- Those willing to provide written informed consent and comply with protocol requirement.

EXCLUSION CRITERIA

- Other concomitant illness like malaria, enteric fever, chikungunya etc. judged by history and physical examination and investigations based.
- Subjects unwilling to consent for the study.

Diagnosis of dengue infection

The diagnosis of DF patients was primarily based on clinical examination and was confirmed by using kit-based solid phase dengue NS1Ag+Ab Duo test immuno-chromatographic assay, one step assay designed to detect both dengue virus NS1 antigen and differential IgM/IgG antibodies to dengue virus in human blood (RapiGEN BIOCREDIT Dengue NS1Ag+Ab Duo Test, Korea) as per manufacturer's instructions.

Proteomic analysis was done in following manner:

- Comparative proteomic analysis of dengue and controls (2DE, 2D-DIGEand QTOF LCMS/MS
- Proteins networks and functional analysis
- Multivariate statistical analysis

Proteomic Analysis

Amongst total 26 enrolled cases of severe dengue (DHF/DSS), (n=26) were sent to IIT Bombay for proteomics analysis. A comparison between severe dengue (DHF/DSS) compared to Healthy controls (HC). HC were defined as adult of same age group, environment, race and disease free.

Plasma separation at PBM Hospital Bikaner

Blood samples were drawn in EDTA vials from all adults included in this study after written and informed consent. Immediately after blood collection, the tubes were kept on ice for 30 minute and then centrifuged at 2000 rpm for 10 minute. After centrifugation blood samples was divided in two parts upper yellow color liquid which was plasma and lower red color were cells and plasma was collected in sample aliquot and stored at - 80^{0}C.

Performed at Indian Institute of Technology, Bombay, Powai, Mumbai as following:

Sample preparation and depletion of abundant protein

Crude plasma was diluted four times with phosphate buffer (pH 7.4) and subjected to gentle sonication with the following settings: 8 cycles of 5 sec pulse; 30 sec gap in between; at 25% amplitude using Sonics vibra cell sonicator.22 proteins contribute to approximately 99% of the protein content in serum and plasma[77]. With the use of depletion column, these high abundant proteins could be removed for the detection of low abundant proteins. In this manner, two high abundant proteins (albumin and IgG) were removed using Albumin &IgG Depletion spin trap (GE Healthcare- 28-9480-20) as per manufacturer's instruction.

Protein extraction by TCA-acetone precipitation

Following depletion, protein extraction was carried out using TCA-acetone precipitation. Slight modifications to the protocols[6] were made for extraction from the plasma samples. In brief, dilution of the samples was carried out using chilled acetone containing 10% w/v of TCA. The mixture was vortexed for 30 seconds and incubated at -20°C for 16 hours. This mixture was then centrifuged at 15000 rpm for 30 minutes at 4°C. To the pellet 1 ml of chilled acetone was added to wash away the remaining TCA and also to the supernatant to completely precipitate the protein. This was followed by vortexing for 30 seconds and incubation at -20°C for 30 mins. The tubes were then centrifuged at 15000 rpm for 20 mins at 4°C. The pellet fraction was washed once more as described and dissolved in Rehydration buffer (RHB) [2 M thiourea, 8 M of urea, 2% (w/v) CHAPS, 0.002% BPB and 0.5 % (v/v) carrier ampholyte]. Sample clean up was performed using 2D clean-up kit by GE Healthcare as per manufacturer's instructions to remove salts, detergents, nucleic acids and lipids that may interfere in the downstream processes.

STATISTICALANALYSIS

Collected data were transferred into SPSS version 17.0 and were analyzed with the help of frequency tables, percentage and appropriate statistical test wherever applicable.

Management

Cases were managed as per standard treatment protocol put forward by WHO (2009).

RESULTS

The present study was conducted in the Department of Medicine, S.P. Medical College and P.B.M. Associated Group of Hospitals, Bikaner.

In present study, 100% cases had IgM positive while 69.2% cases had NS1 positive. No case had IgG positive. Mean age in DHF group was 30.4812.58 and in DSS group mean age was 25.0012.39 years and this difference was found statistically insignificant (p>0.05).

Most common clinical manifestation was fever, abdominal pain and myalgia (100%) while rash was present in 96.2%, vomiting and headache were present in 92.3% cases each and least common clinical manifestation was retro-orbital pain (88.5%). Gum bleeding was present in 90.5% cases, epistaxis was present in 66.7% petechiae was present in 52.4%, hematuria was present in 33.3% cases, hematemesis was present in 14.3% of cases and melena was present in 9.5% cases while in DSS group, 100% cases had gum bleeding, 80% cases had epistaxis, 60% cases had petechiae, 40% each cases had hematemesis and hematuria. Purpura and subconjunctival haemorrhage was not found in any case (p>0.05).

On day 1, mean haemoglobin in DHF group was 7.560.34 and in DSS group it was 8.320.48 mg/dl (p<0.01), on day 2, mean haemoglobin in DHF group was 7.680.31 and in DSS group it was 8.52045 (p<0.01), on day 3 mean haemoglobin was 7.961.40 mg/dl and in DSS group it was 8.760.41 (p<0.001) while on day 4, mean haemoglobin in DHF group was 8.180.13 and in DSS group it was 8.970.43 (p<0.001).

Asictes was present in 15 cases and out of them 13 and 2 cases belonged to DHF and DSS group respectively, bilateral pleural effusion was found in total 13 cases and out of them 10 and 3 cases belonged to DHF and DSS groups respectively, GB wall edema was present in 5 cases and out of them 4 and 1 case belonged to DHF and DSS group respectively while hepatomegaly was found in 2 cases and they both belonged to DHF group (p>0.05 in all). According to outcome, 20 cases were cured successfully while 6 cases discharge on request. Patients were on telephonically follow up for one week, there was no mortality.

Proteomic analysis in severe dengue patients (DHF/DSS) shows down regulation of three significant proteins CD-44 antigen, complement component C8 beta chain and leucine-rich alpha-2-glycoprotein as compared to healthy controls.

DISCUSSION

Dengue is endemic in SEAR (South East Asia Region) including India. In India, dengue epidemics are becoming more frequent. Every year cases are spreading to newer geographical area. All four dengue virus serotype have been isolated from different parts of the country. India contributed 6-9% of total cases in SEAR countries between 2009 and 2011, which has increased to 19% in 2013. Dengue is one disease entity with different clinical presentations and often with unpredictable clinical evaluation and outcome.

The mechanisms that trigger transition from mild DF to more life threatening DHF are poorly understood, hampering early classification of dengue patients who will progress to DHF. This not only delays treatment but frequently result in the over hospitalisation of the patients, contributing significantly to the financial burden imposed by dengue[7,8].The availability of reliable markers that predict DHF during the early stage of infection could be useful in triaging patients for management.

Out of total 26 cases, 21 (80.7%) had dengue haemorrhagic fever (DHF) while 5 (19.3%) cases had dengue shock syndrome (DSS). The results were similar to study conducted by China et al[9] where a higher percentage of cases had DHF as compared to DSS. All the cases (26) were IgM positive while 18 cases were NS1 positive. No case was found to be IgG positive.

In the present study, majority (100%) of the patients presented with fever, abdominal pain and myalgia. The other symptoms observed were rash (96.2%), vomiting and headache (92.3% each), retro- orbital pain (88.5%). It was similar to study conducted by Kumar et al[10] which showed fever (85%), headache (73%), arthralgia (52%), retro orbital pain (70%), abdominal pain (54%), rash (16%), vomiting (70%) and diarrhoea (19%). The symptoms observed in the study by Laulet al[11] were fever (100%), headache (87%), bodyache (86%), retro orbital pain (58%), rashes (21%).

In our study, among the bleeding manifestations most common was gum bleeding with 92.3% cases followed by epistaxis (69.2%), petechaie (53.8%), hematuria (34.6%), hematemesis (19.2%), melena (7.7%) while no cases had purpura and subconjuctival haemorrhage. All these parameters had an insignificant correlation when we compared them between DHF and DSS groups ($p>0.05$). The results were in accordance to Zhang et al[12] study where the gum bleeding was the most common bleeding manifestation followed by epistaxis.

Haemoglobin levels in dengue fever corresponds to hematocrit level and hemoconcentration. There was fall in Hb in patients with bleeding manifestation. In this study we observed the pattern of Hb level over the entire duration of admission. Mean hemoglobin levels on day 1 of admission (day 3-4 of fever) were found to be 8.320.48 in DSS group while it was 7.560.34 mg/dl in DHF group ($p<0.01$). The mean haemoglobin values increased on subsequent days in both DSS and DHF groups with the values being 8.970.43 and 8.180.13 respectively ($p<0.001$) on day 4 of admission. The mean values in DSS patients were higher on all the days as compared to DHF patients. The difference became statistically significant on day 3 and 4 of admission. Hemoglobin levels were found to be lower during initial days of admission and was significantly lower in DHF group of diseased population. The reason was hemodilution due to plasma leakage and hemorrhagic tendencies in DHF whose maximum incidence is during 4-6 day of fever. Similar results were observed in Mehrotrae al[13] study who showed that on day 4 of fever 52.78% patients had normal haemoglobin which increased to 57.14% on day 7 and 60% on day 10 of fever.

In the present study, ascites was the most common USG finding with 57.7% cases followed by bilateral pleural effusion (50%), GB wall edema (19.2%) and hepatomegaly (7.7%). Ascites was more common in DHF patients while pleural effusion was more common in DSS. The results were in accordance to the study by Khurramet al[14] who observed that ascites was the most frequently noted USG finding followed by pleural effusion and GB wall edema.

In our study, according to outcome, 20 (76.9%) cases were cured successfully while 6 (23.1%) cases were discharged on request. Patients were on follow up telephonically for one week, there was no mortality.

In our study, we validated the human serum proteome alterations that occurred due to severe dengue fever and had altered expression levels associated with coagulation and complement cascade, and inflammation-mediated acute phase signalling. To the best of our knowledge, we report here the first comprehensive analysis validating the significant protein markers seen in severe dengue fever patients in Bikaner (North-west Rajasthan). We have optimized a number of differentially expressed serum proteins using Selected Reaction Monitoring Assay and performed analysis validating the proteomic markers in severe dengue patients. Three significant proteins CD44 antigen, Complement component C8 beta chain, Leucine-rich alpha-2-glycoprotein were analysed in our study. CD44 is an adhesion molecule for extracellular matrix proteins and is a cytotoxicity marker present on activated NK cells. Complement component C8 beta chain is a constituent of the membrane attack complex (MAC) that plays a key role in the innate and adaptive immune response by forming pores in the plasma membrane of target cells. Leucine-rich alpha-2-glycoprotein is a secretory type1 acute phase protein whose expression is upregulated by the mediators of acute phase response and its level increases during infections and cancer.

We observed that these markers are down-regulated in the sera of severe dengue patients (DHF/DSS) as compared to healthy controls that samples were taken from same institution for proteomic analysis of severe dengue patients[15] because of fold change less <1. Fold change values were 0.987409, 0.987244 and 0.984124 in CD44 antigen, Complement component C8 beta chain (CO8B) and Leucine-rich alpha-2-glycoprotein (A2GL) respectively. Thus, it was observed that proteins CD44 antigen, Complement component C8 beta chain, Leucine-rich alpha-2-glycoprotein are predictive biomarkers of severe dengue fever (DHF/DSS).

Chun-Yu-Lin et al[16]study observed that highly circulating level of viral protein NS1 is indicative of disease severity. Its exposure decreased the expression of CD44 in differentiating endothelial cells impairing the interity of vessel like structures. This study supported our result of downregulation of CD44 antigen.

In study by Flores-Mendoza et al[17], it was observed that IL 10 and socs3 are predictive biomarkers of DHF while Manchala et al[18] showed the prognostic role of apolipoprotein A-I (APO A-I) in dengue fever. Jadhav et al[15] found significant elevation in IL-1 RA, IL-7, TNF-α, MCP1-MCAF and MIP-1β levels in DHF patients. Mapalagamageet al[19]showed that serum NOx may be used as a potential prognostic marker of DHF in patients presenting with DF in the early stage (on day 3 of fever) of the disease.

Since our sample size was small, further studies on these proteins are needed to better understand the cellular response to dengue virus infection and for the identification and validation of predictive biomarkers of severe dengue fever which would help to develop new diagnostic tools for early and timely detection of severe dengue infection.

CONCLUSION

In present study revealed that validating 26 serum samples used for optimization by using selected reaction monitoring. Three significant proteins, CD44 Antigen, Complement component C8 beta chain, Leucine-rich alpha-2-glycoprotein are downregulated in severe dengue (DHF/DSS) patients as compared to healthy controls. These are predictive biomarkers of severe dengue fever (DHF/DSS).

Since our sample size was small in number, further studies on these large sample size are needed to better understand the cellular response to dengue virus infection and validation of predictive biomarker of severe dengue fever which would help to develop new diagnostic tools for early detection of severity.

Table 1 Distribution of cases according to dengue agglutination test

Dengue Agglutination Test	DHF		DSS		Total		X^2	p
	No.	%	No.	%	No.	%		
IgM	21	100	5	100	26	100	-	-
IgG	0	-	0	-	0	-	-	-
NS1	15	71.4	3	60.0	18	69.2	0.248	0.619

Table 2 Distribution of cases according to clinical manifestation

Clinical Manifestations	DHF		DSS		Total		X^2	p
	No.	%	No.	%	No.	%		
Fever	21	100	5	100	26	100	-	-
Abdominal Pain	21	100	5	100	26	100	-	-
Myalgia	21	100	5	100	26	100	-	-
Rash	20	95.2	5	100	25	96.2	0.248	0.619
Vomiting	19	90.5	5	100	24	92.3	0.516	0.473
Headache	19	90.5	5	100	24	92.3	0.516	0.473
Retro-orbital Pain	18	85.7	5	100	23	88.5	0.807	0.369

Table 3 Distribution of cases according to bleeding manifestations

Bleeding Manifestations	DHF		DSS		Total		X^2	p
	No.	%	No.	%	No.	%		
Gum Bleeding	19	90.5	5	100	24	92.3	0.516	0.473
Epistaxis	14	66.7	4	80.0	18	69.2	0.337	0.562
Petechiae	11	52.4	3	60.0	14	53.8	0.094	0.759
Hematuria	7	33.3	2	40.0	9	34.6	0.292	0.864
Hematemesis	3	14.3	2	40.0	5	19.2	1.857	0.395
Melena	2	9.5	0	-	2	7.7	0.516	0.473
Purpura	0	-	0	-	0	-	-	-
Subconjunctival Haemorrhage	0	-	0	-	0	-	-	-

Table 4 Distribution of cases according to sensorium

Sensorium	DHF		DSS		Total	
	No.	%	No.	%	No.	%
Altered	1	4.8	0	-	1	3.8
Normal	20	95.2	5	100	25	96.2
Total	21	100	5	100	26	100
X^2	0.248					
p	0.619					

Table 5 Distribution of cases according to haemoglobin

Haemoglobin	DHF		DSS		t	p
	Mean	SD	Mean	SD		
Day 1	7.56	0.34	8.32	0.48	3.288	0.003
Day 2	7.68	0.31	8.52	0.45	3.885	0.001
Day 3	7.96	1.40	8.76	0.41	4.231	<0.001
Day 4	8.18	0.13	8.97	0.43	4.029	<0.001

Table 6 Distribution of cases according to outcome

Outcome	DHF		DSS		Total	
	No.	%	No.	%	No.	%
Cured	15	71.4	5	100	20	76.9
Discharge on Request	6	28.6	0	-	6	23.1
Total	21	100	5	100	26	100
X^2	1.857					
P	0.173					

Table 7

S.no.	Protein ID	Protein name	HC	DHF/DSS	FC
1	P16070	CD44 antigen	2.684922	2.651115	0.987409
2	P07358	Complement component C8 beta chain	5.307983	5.240276	0.987244
3	P02750	Leucine-rich alpha-2-glycoprotein	7.421469	7.303648	0.984124

Table 8 List of proteins taken for optimization of the SRM assay

sp|P02750|A2GL_HUMAN	Leucine-rich alpha-2-glycoprotein OS=Homo sapiens OX=9606 GN=LRG1 PE=1 SV=2	P02750
sp|P04004|VTNC_HUMAN	Vitronectin OS=Homo sapiens OX=9606 GN=VTN PE=1 SV=1	P04004
sp|P00738|HPT_HUMAN	Haptoglobin OS=Homo sapiens OX=9606 GN=HP PE=1 SV=1	P00738
sp|Q08380|LG3BP_HUMAN	Galectin-3-binding protein OS=Homo sapiens OX=9606 GN=LGALS3BP PE=1 SV=1	Q08380
sp|P0DJI8|SAA1_HUMAN	Serum amyloid A-1 protein OS=Homo sapiens OX=9606 GN=SAA1 PE=1 SV=1	P0DJI8
sp|P17936|IBP3_HUMAN	Insulin-like growth factor-binding protein 3 OS=Homo sapiens OX=9606 GN=IGFBP3 PE=1 SV=2	P17936
tr|D6RF35|D6RF35_HUMAN	Vitamin D-binding protein OS=Homo sapiens OX=9606 GN=GC PE=1 SV=1	D6RF35
sp|P49747|COMP_HUMAN	Cartilage oligomeric matrix protein OS=Homo sapiens OX=9606 GN=COMP PE=1 SV=2	P49747
sp|P01023|A2MG_HUMAN	Alpha-2-macroglobulin OS=Homo sapiens OX=9606 GN=A2M PE=1 SV=3	P01023
sp|P10909|CLUS_HUMAN	Clusterin OS=Homo sapiens OX=9606 GN=CLU PE=1 SV=1	P10909
sp|P02647|APOA1_HUMAN	Apolipoprotein A-I OS=Homo sapiens OX=9606 GN=APOA1 PE=1 SV=1	P02647
sp|P06396|GELS_HUMAN	Gelsolin OS=Homo sapiens OX=9606 GN=GSN PE=1 SV=1	P06396
sp|P09871|C1S_HUMAN	Complement C1s subcomponent OS=Homo sapiens OX=9606 GN=C1S PE=1 SV=1	P09871
sp|P00450|CERU_HUMAN	Ceruloplasmin OS=Homo sapiens OX=9606 GN=CP PE=1 SV=1	P00450
sp|P04180|LCAT_HUMAN	Phosphatidylcholine-sterol acyltransferase OS=Homo sapiens OX=9606 GN=LCAT PE=1 SV=1	P04180
sp|P07358|CO8B_HUMAN	Complement component C8 beta chain OS=Homo sapiens OX=9606 GN=C8B PE=1 SV=3	P07358
sp|Q9UGM5|FETUB_HUMAN	Fetuin-B OS=Homo sapiens OX=9606 GN=FETUB PE=1 SV=2	Q9UGM5
sp|O75636|FCN3_HUMAN	Ficolin-3 OS=Homo sapiens OX=9606 GN=FCN3 PE=1 SV=2	O75636
sp|P01008|ANT3_HUMAN	Antithrombin-III OS=Homo sapiens OX=9606 GN=SERPINC1 PE=1 SV=1	P01008
sp|P02760|AMBP_HUMAN	Protein AMBP OS=Homo sapiens OX=9606 GN=AMBP PE=1 SV=1	P02760
sp|P19320|VCAM1_HUMAN	Vascular cell adhesion protein 1 OS=Homo sapiens OX=9606 GN=VCAM1 PE=1 SV=1	P19320
sp|P16070|CD44_HUMAN	CD44 antigen OS=Homo sapiens OX=9606 GN=CD44 PE=1 SV=3	P16070
sp|Q9NZP8|C1RL_HUMAN	Complement C1r subcomponent-like protein OS=Homo sapiens OX=9606 GN=C1RL PE=1 SV=2	Q9NZP8
sp|P02747|C1QC_HUMAN	Complement C1q subcomponent subunit C OS=Homo sapiens OX=9606 GN=C1QC PE=1 SV=3	P02747
sp|P25311|ZA2G_HUMAN	Zinc-alpha-2-glycoprotein OS=Homo sapiens OX=9606 GN=AZGP1 PE=1 SV=2	P25311
sp|P04217|A1BG_HUMAN	Alpha-1B-glycoprotein OS=Homo sapiens OX=9606 GN=A1BG PE=1 SV=4	P04217

REFERENCES

1. Halstead SB, Suaya JA, Shepard DS. The burden of dengue infection. Lancet 2007;369:1410–1411.
2. Wu SJ, Grouard-Vogel G, Sun W, Mascola JR, Brachtel E, et al. Human skin Langerhans cells are targets of dengue virus infection. Nat Med 2000; 6: 816–820.
3. Guzman MG, Kouri G. Dengue : An Update. Lancet Infect Dis 2002; 2:33-42.
4. World Health Organization. First report on neglected tropical diseases: working to overcome the global impact of neglected tropical diseases. Geneva: World Health Organization; 2010.
5. World Health Organization. Comprehensive guidelines for prevention and control of dengue and dengue hemorrhagic fever. New Delhi: WHO, SEARO; 2011: revised and expanded edition.
6. Chen YY, Lin SY, Yeh YY, Hsiao HH, Wu CY, Chen ST, Wang AHJ. A modified protein precipitation procedure for efficient removal of albumin from serum. Electrophoresis. 2005; 26(11): 2117-2127.
7. Clark DV, Mammen MP, Nisalak A, Puthimethee V, Endy TP. Economic impact of dengue fever/dengue haemorrhagic fever in Thailand at the family and population levels. The American journal of tropical medicine and hygiene. 2005; 1:72(6):786-91.
8. Suaya JA, Shepard DS, Siqueira JB, Martelli CT, Lum LC, et al. Cost of dengue cases in eight countries in the Americas and Asia: a prospective study. Am J Trop Med Hyg 2009; 80: 846–855.
9. Chinna RS, Goyal O, Chhina D. Liver function test in patients with dengue viral infection. Dengue Bull 2008; 32:346.
10. Kumar S, Bhandiwad C, Lakhiwal R, Singh CP, Sharma N, Chhimpa A. Study of dengue outbreak in North West zone of Rajasthan, India. Int J Res Med Sci 2018;6(9)2905-8.
11. Laul A, Laul P, Merugumala V, Pathak R, Miglani U, Saxena P. Clinical profiles of dengue infection during an outbreak in Northern India. J Trop Med 2016; 5917934.
12. Zhang H, Zhou YP, Peng HJ, Zhang XH, Zhou FY, Liu ZH, Chen XG. Predictive symptoms and signs of sevee dengue disease for patients with dengue fever:A meta analysis. Bio Med Res Int 2014; 359308.
13. Mehrotra S, Singh M, Kumar A, Misra R. Hematological parameters in dengue fever. Int J Advanc Res 2018; 6(3):1247-54.
14. Khurram M, Qayyum W, Umar M, Jawad M, Mumtaz S, Khaar HTB. Ultrasonographic pattern of plasma leak in dengue haemorrhagic fever. J Pak Med Assoc 2016; 260-4.
15. Jadhav M, Nayak M, Kumar S, Venkatesh A, Patel SK, Kumar V. Clinical Proteomics and Cytokine Profiling for Dengue Fever Disease Severity Biomarkers. OMICS. 2017; 21(11):665-677.
16. Lin CY, Kolliopoulos C, Huang CH, Tenhunen J, Heldin CH, Chen YH et al. High levels of seumhyaluronan is an early predictor of dengue warning signs and perturbs vascular integrity. E Bio Med 2019; 48L425-441.
17. Flores-Mendoza LK, Estrada-Jimenez T, Sedeno-Monge V, Moreno M, Manjarrez MDC et al. IL-10 and socs3 are predictive biomarkes of dengue hemorrhagic fever. Medications of Inflammation 2017; 2017: 5197592.
18. Manchala NR, Dungdung R, Pilankatta R. Proeomic analysis reveals the enhancement of human serum apolipoprotein A-1 (APO-1) in individuals infected with multiple dengue virus serotypes. Trop Med IntHlth 2017; 22(10):1334-42.
19. Mapalagamage M, Handunnetti S, Premawansa G, Thillainathan S, Fernando T, KanapathippillaiK et al. Is total serum nitric and nitrate (NOx) level in dengue patients a potential prognostic marker of dengue hemorrhagic fever? Dis Mar 2018; 5328681:9.