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Assessment of Biochemical and Antioxidative Status in Patients Suffering from Dengue Fever*

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Summary: A multi-centred study was designed to collect dengue epidemiologic data from government and registered private hospitals/clinics and maintained archive of frozen specimens in bio-bank to be used for future dengue epidemic control program, and assess the epidemiology of dengue fever (DF) by evaluating biochemical and oxidative status of patients. ELISA IgM antibodies test was done to confirm DF. From August 2010 to December 2011, 101 confirmed blood samples of DF patients referred to pathology lab of Jinnah Hospital Lahore were subjected to the epidemiologic assessment by evaluating the biochemical and physiological indices and alterations of circulating antioxidants. Clinical features of DF patients and effect of fever on blood components and serum proteins of liver were recorded. The hospital stay in DF, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) showed significant difference. Significant increases in serum alanine amino transferase (ALT) (P=0.000), aspartate amino transferase (AST) (P=0.000), alkaline phosphatase (ALP) (P=0.000), malondialdehyde (MDA) along with significant decreases in total protein (TP) (P=0.000), reduced glutathione (GSH) (P=0.000), superoxide dismutase (SOD), catalase (CAT) (P=0.000), and sialic acid contents (P=0.016) were observed. A positive correlation existed between bound sialic acid levels, liver enzymes and circulating antioxidants (r=0.656, P=0.016). In the present study, alterations of circulating antioxidants in DF suggest that DF might be a metabolic response to an acute, self-limiting tropical viral infection, and a consequence of the viral inflammatory process.

Key words: dengue fever; alanine amino transferase; aspartate amino transferase; malondialdehyde; superoxide dismutase; catalase

Dengue fever (DF) is a viral disease transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes[1]. Like many other diseases, the DF also shows the change in the oxidative stress (OS) in the patients. Unbalancing of oxidants and antioxidants results in cell damage and production of highly reactive and unstable free radicals. OS shows its effect by inducing boosted metabolism of fatty acids in the form of lipid peroxidation (LPO) and producing biologically active molecules like malondialdehyde (MDA). Such molecules may lead to fibrosis. The cell, on contrary, has protective system against the OS to nullify the effects of oxidants by antioxidants like glutathione (GSH), vitamin E and vitamin C[2, 3].

DF and dengue hemorrhagic fever (DHF) have been a major problem for human community after the 2nd World War particularly in the region of Southeast Asia. DF is usually a self-limiting viral disease; on the other hand, DHF is a serious and problematic infection that shows high morbidity and mortality[4].

It has been observed that intracellular redox balance is responsible for the promotion of viral infection and viral-induced diseases. Reactive oxygen species (ROS) are involved in viral infected cells and eradicated by antioxidants. GSH, catalase (CAT), thioredoxin (Trx) and superoxide dismutase (SOD) produce the environment in the cell that acts against the oxidants and keep the reducing environment in the cell[5]. OS due to effect of dengue infection on protein-bound sulphydryls in human plasma is not well understood[6]. In dengue patients, increased

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levels of total antioxidant status (TAS) like SOD, GSH, and LPO have been observed, and decreased levels of glutathione peroxidase (GPx) and total hydroperoxides (THP) have been observed as compared to normal[7].

1 MATERIALS AND METHODS

1.1 Subjects

A total of 111 patients clinically suspected for DF from different areas of Lahore who went to Jinnah Hospital Lahore from August 2010 to December 2011 were tested for dengue infection. Out of these, 101 (90%) were confirmed serologically reactive for IgM antibody, reflecting recent dengue infection. Out of 9 administrative towns of Lahore, maximum dengue cases (5%) were diagnosed from Samanabad town followed by Dataganj Baksh, AllamaIqbal, Gulberg, Shalimar, Ravi, AzizBhatti, Nishtar and Wagha towns. Reactive dengue cases were reported from every town of Lahore with varying number of union councils involved in each town. The mean age of the dengue patients was 40.44±19.69 years and 90% belonged to 20–40 years age groups. More males (52%) were affected than females (48%).

1.1.1 Experimental Site Diagnosis of specimen and relevant patient data collection were done at Department of Pathology, Jinnah Hospital Lahore, Pakistan and biochemical test was conducted at Institute of Molecular Biology and Biotechnology, University of Lahore, Pakistan.

1.1.2 Inclusion and Exclusion Criteria Suspected dengue patients, diagnosed with DF or DHF, in whom the results of complete blood count (CBC) were available were included in this study. Patients with a history of hepatitis C were excluded.

1.2 Chemical Analysis

Aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) were evaluated by commercial kits. MDA was estimated by Ohkawa method[8]. Total protein (TP) was estimated by Lowry method[9]. GSH was evaluated by Beutler method[10]. SOD was assessed by Kakkar method[11]. CAT was estimated by Aebi method[12].

1.3 Statistical Analysis

The difference and correlations between the values of different parameters of dengue patients and those of healthy controls were determined by statistical package of SPSS var18. The significance of the difference between populations was taken at least $P<0.05$. Least significant difference (LSD) was calculated by COSTAT.

2 RESULTS

2.1 Effects of DF on Haematological Characteristics

Table 1 shows effect of DF on blood components and serum proteins of liver. Level of significance has also been calculated for dengue patients against healthy controls. The results of haematocrit in dengue patients showed that there was significant change as compared to controls ($P<0.000$). The increase in haematocrit level in serum showed severe dehydration, which might be due to hypersplenism. The raise in level of haematocrit suggests that there was severe imbalance in homeostasis, which caused vomiting and diarrhoea, leading to dehydration in dengue patients. The platelet count presented in table 1 was significantly reduced in patients with DF [(37.38±24.77)×10^3/μL] as compared with the controls [(348.8±69.85)×10^3/μL] ($P<0.000$). The decreased levels of platelet count suggested that there was severe thrombocytopenia which caused bleeding from gums and led to DHF and dengue shock syndrome (DSS). Table 1 also shows a significant decrease in white blood cells (WBCs) levels in serum of dengue patients as compared to healthy individuals, resulting in leucopenia.

Table 1 Effect of DF on blood components and serum proteins of liver

Content	Controls	Dengue patients	*P* value
Age	42.00±24.40	40.44±19.70	
Hematocrit (%)	40.236±1.8	43.95±5.10	0.000
Platelets (×10^3/μL)	348.8±69.85	37.38±24.77	0.000
WBC (×10^3/μL)	8.37±1.48	4.86±2.43	0.000
MCV	91.88±2.81	89.60±8.38	0.396
RBC (×10^6/μL)	4.55±0.76	4.28±0.56	0.998
Hb (g/dL)	13.03±2.17	12.76±2.12	0.700
Plasma bilirubin (mg/dL)	0.49±0.185	0.98±0.39	0.000
ALT or SGPT (U/L)	23.40±5.69	70.27±26.61	0.000
AST or SGOT (U/L)	26.00±5.55	47.84±17.23	0.000
ALP (U/L)	29.00±4.73	247±75.46	0.000
TP (mg/dL)	5.00±0.87	7.7±1.76	0.000
MDA (μmol/mL)	3.00±1.36	3.46±1.14	0.237
SOD (ng/mL)	0.92±1.79	0.19±0.14	0.231
GSH (mg/dL)	9.82±1.32	2.04±0.84	0.000
CAT (μmol/mol of protein)	4.29±0.83	0.80±0.94	0.000
Sialic acid (μg/mL)	2.71±130.40	39.9±43.9	0.016

The data regarding ALT, AST, plasma bilirubin, ALP and total proteins presented in table 1 shows significant change of liver functions in DF. A raising trend was noted for ALT, AST, plasma bilirubin, total proteins and ALP in dengue patients as compared to healthy individuals, reflecting acute hepatic failure. Kuo *et al*[13] has reported higher bleeding episodes in those who had high levels of AST and ALT. Another study[14] has also reported significantly higher spontaneous bleeding episodes in patients with elevated ALT, and ALP was also

found to be significantly higher. AST and ALT were elevated in DHF patients with gastrointestinal haemorrhage.

The level of MDA, a by-product of lipid peroxidation, was higher in dengue patients (3.46±1.14 μmol/mL) than in controls (3.00±1.36 μmol/mL). The significant increase in GSH was recorded in dengue patients (9.28±1.32 mg/dL) as compared to controls (2.04±0.84 mg/dL) (P<0.000). A significant decrease in SOD level was found in dengue patients (0.19±0.14 ng/mL) as compared with that in controls (0.92±1.79 ng/mL). The decreased level of SOD was attributed to high oxidative stress and production of a large number of ROS, and to neutralize these ROS, SOD was consumed. CAT value for healthy controls was 4.29±0.83 μmol/mol of protein, and that was reduced to 0.80±0.94 μmol/mol of protein in dengue patients. Large number of H_2O_2 ions was produced in dengue patients, and CAT was consumed to neutralize them.

Table 2 lists demographic features during dengue episode in Lahore-Pakistan. Patients were divided into different age groups and their frequency was reported for gender (male=54, and female=47), income status (high=40, and low=61), locality (urban=87, and rural=14) and smoking habits (male smokers=28, and male non-smokers=28). Table 3 represents attack rate of dengue in all affected Union Councils of 9 towns of Lahore during 2010–2011.

Table 2 Demographic factors during Dengue episode (2011) in Lahore-Pakistan

Age groups (years)	Gender		Income status		Locality		Smoking habit*	
	Male	Female	High income	Low income	Urban	Rural	Smokers	Non-smokers
0–10	1	3	2	2	4		1	0
11–20	10	3	3	10	11	2	6	4
21–30	12	10	9	13	18	4	6	6
31–40	7	7	4	10	12	2	3	4
41–50	5	7	6	6	10	2	2	3
51–60	14	10	14	10	21	3	7	7
61–70	2	3	1	4	5		1	1
71–80	2	1	1	3	2	1	2	0
81–90	1	3	0	3	4		0	1
Total	54	47	40	61	87	14	28	26

*Data for smoking habit represent male patients only.

Table 3 Attack rate of dengue in all affected Union Councils of 9 towns of Lahore during 2010–2011

Areas	Total No. of Union councils	Population at risk	Percentage of cases (%)	Attack (rate/million)
Ravi Town	19	996 708	9.93	9.8
Shalimar Town	17	862 435	7.30	6.2
Wagah Town	12	647 181	5.56	3.55
Aziz Bhatti Town	13	719 566	5.00	8.05
Data Gunj Bakhsh Town	18	1109 262	7.30	12.5
Gulberg	15	667 108	8.09	5.33
Samanabad Town	19	949 558	4.73	4.46
Allama Iqbal Town	19	1037 692	1.04	10.79
Nishtar Town	18	948 382	1.23	11.6
Rural areas	–	–		
Total/average		7937 892/881 988	50.18/5.57	72.28/8.03

2.2 Effects of DF on Liver Functions (ALT, AST, ALP, TP)

ALT is an important liver enzyme used as a biomarker for detection of liver injury and severity for DF. The time dependent study of ALT showed a significant increase in ALT (49.20±2.23 U/L) during DF as compared with healthy controls (20.70±3.55 U/L), independent of gender type. The value of ALT was observed statistically different on day-to-day basis; it was 32.53±2.49, 48.33±1.26, 41.00±3.26 and 37.00±3.2 U/L on 3rd, 5th, 7th and 9th day, respectively (table 4). Maximum liver damage was observed on 5th day of viremia. The ALT gradually increased up to 48.33±1.26 U/L as fever progressed and got back to normal after 7–14 days of fever. The results suggested that DF caused elevation of ALT.

2.3 Effects of DF on Antioxidant Status (GSH, MDA, SOD, CAT and Platelets)

In order to investigate the effect of dengue infection on the GSH level, the GSH levels were assayed at different days post-infection. The dengue infection caused a time-dependent alteration in GSH levels: 7.82±2.1, 6.87±2.4, 5.40±3.35, and 4.65±4.02 mg/dL on 3rd, 5th, 7th and 9th day, respectively (table 5). The data showed that the GSH level decreased from normal (9.92±0.98 mg/dL) on daily basis to 4.65±4.02 mg/dL on 9th day of infection. GSH tended to recover once fever was over. The results also indicated that there was no significant difference in GSH level between male (4.62±2.19 mg/dL) and female (4.00±2.10 mg/dL) patients but both were significantly different from controls. These results indi-

cated that dengue infection could cause decrease in GSH level.

Table 4 Effect of DF duration on ALT levels

Source	Sum of square	Degree of freedom	Mean square	Frequency	*P*-value
Sex (S)	10849.03	2	5424.51	185.24	0.000***
Duration (D)	2023.11	3	674.37	23.03	0.000***
Sex X duration	1324.43	6	220.73	7.53	0.000***
Error	1405.6	48	29.28		
Total	15602.18	59			
Duncan's Multiple Range test					
	3rd day	5th day	7th day	9th day	LSD=3.44
Control	22.00±4.52	20.00±3.53	20.20±2.95	20.60±3.91	20.70±3.55[b]
Male	36.20±3.27	63.60±6.11	50.60±6.69	46.40±6.73	49.20±3.43[a]
Female	39.23±2.60	61.40±3.91	52.20±8.28	44.00±8.09	49.25±2.33[a]
LSD=3.97	32.53±2.49[d]	48.33±1.26[a]	41.00±3.26[b]	37.00±3.26[c]	

***: highly significant; Alphabets (a, b, c and d) are represented by software for statistical difference among the mean value of different group.

Table 5 Effect of dengue fever duration on GSH levels

Source	Sum of square	Degree of freedom	Mean square	Frequency	*P*-value
Sex (S)	423.57	2	211.7	164.24	0.000***
Duration (D)	91.47	3	30.49	23.64	0.000***
Sex X duration	47.19	6	7.86	6.10	0.000***
Error	61.89	48	1.28		
Total	624.13	59			
Duncan's Multiple Range test					
	3rd day	5th day	7th day	9th day	LSD=0.72
Control	10.03±1.48	9.82±1.13	9.82±0.54	10.04±0.85	9.92±0.98[a]
Male	7.3±0.92	5.63±0.88	3.47±0.78	2.09±0.85	4.62±2.19[b]
Female	6.1±1.75	5.1±0.15	2.92±1.29	1.83±0.95	4.00±2.10[b]
LSD=0.83	7.82±2.14[a]	6.87±2.43[b]	5.40±3.35[c]	4.65±4.02[c]	

***: highly significant; Alphabets (a, b, c and d) are represented by software for statistical difference among the mean value of different group.

The lipid peroxidation was reflected by MDA level at different days of infection and the level was found to be low initially, 1.87±0.29 μmol/mL on 3rd day and 2.09±0.62 μmol/mL on 5th day of infection (table 6). The level of MDA increased gradually, 2.87±1.12 μmol/mL on 7th day and highest (3.66±1.171 μmol/mL) on 9th day of infection as compared with the controls (1.58±0.55 μmol/mL). This is probably due to cell lysis by the increased generation of ROS. The result also showed that there was statistical difference in lipid peroxidation between males and females (2.94±0.98 μmol/mL *vs*. 3.44±1.33 μmol/mL). The raise in MDA level is caused by cell membrane damage, as cell membrane is made up of lipid bilayer; hence, the raise in MDA levels is due to increased production in ROS in patients suffering from DF. It is suggested that the raise in MDA is caused by imbalance of total antioxidative system in DF.

In order to analyze the effect of dengue infection on the SOD level, the SOD levels were assayed at different days postinfection. The dengue infection caused a time-dependent alteration in SOD level. The level of SOD decreased as fever progressed, 0.37±0.08, 0.32±0.09, 0.27±0.12, and 0.20±0.14 ng/mL on 3rd, 5th, 7th and 9th day of infection, respectively (table 7). We did not find any significant difference in SOD level between males (0.24±0.10 ng/mL) and females (0.22±0.11 ng/mL), but both were significantly different from controls (0.41±0.07 ng/mL). These results indicated that dengue infection decreased SOD level.

Platelets are part of coagulation. The platelets were measured at different days of fever and platelet count was significantly reduced during DF. Time-dependent changes in platelets counts showed descending pattern, 359.4±64.94 (control) to 238.73±112.95 (3rd day) to 169.26±152.12 on the 9th day of fever (table 8). This is probably due to destructions of platelets by dengue virus. The result also showed that there was statistically significant difference in platelet count between males (140±39.32) and females (98.8±39.22). It is suggested that decrease in platelet count is caused by dengue virus.

Table 6 Effect of DF duration on MDA levels

Source	Sum of square	Degree of freedom	Mean square	Frequency	*P*-value
Sex (S)	40	2	20.31	106.97	0.000***
Duration (D)	29.96	3	9.98	52.58	0.000***
Sex X duration	15.86	6	2.64	13.91	0.000***
Error	9.11	48	0.189		
Total	95.57	59			
Duncan's Multiple Range test					
	3rd day	5th day	7th day	9th day	LSD=0.27
Control	1.67±0.30	1.27±0.11	1.47±0.35	1.58±0.55	1.49±0.37^{c}
Male	1.92±0.33	2.40±0.13	3.19±0.32	4.24±0.69	2.94±0.98^{b}
Female	2.0±0.11	2.6±0.23	3.95±0.41	5.17±0.87	3.44±1.33^{a}
LSD=0.31	1.87±0.29^{c}	2.09±0.62^{c}	2.87±1.12^{b}	3.66±1.71^{a}	

***: highly significant; Alphabets (a, b, c and d) are represented by software for statistical difference among the mean value of different group.

Table 7 Effect of DF duration on SOD levels

Source	Sum of square	Degree of freedom	Mean square	Frequency	*P*-value
Sex (S)	0.42	2	0.21	41.21	0.000***
Duration (D)	0.22	3	0.073	14.26	0.000***
Sex X duration	0.09	6	0.015	2.92	0.0165*
Error	0.24	48	0.00		
Total	0.98	59			
Duncan's Multiple Range test					
	3rd day	5th day	7th day	9th day	LSD=0.04
Control	0.41±0.08	0.41±0.05	0.43±0.07	0.38±0.07	0.41±0.07^{a}
Male	0.36±0.04	0.30±0.07	0.19±0.04	0.11±0.02	0.24±0.10^{b}
Female	0.33±0.09	0.25±0.08	0.19±0.06	0.12±0.08	0.22±0.11^{b}
LSD=0.05	0.37±0.08^{a}	0.32±0.09^{ab}	0.27±0.12^{b}	0.20±0.14^{c}	

***: highly significant; *: significant; Alphabets (a, b, c and d) are represented by software for statistical difference among the mean value of different group.

Table 8 Effect of DF duration on platelets count

Source	Sum of square	Degree of freedom	Mean square	Frequency	*P*-value
Sex (S)	784 974.4	2	39 2487.2	238.47	0.000***
Duration (D)	38 988.26	3	12 996.08	7.89	0.0002***
Sex X duration	20 762.93	6	3460.48	2.10	0.0702
Error	79 000.8	48	1645.85		
Total	923 726.4	59			
Duncan's Multiple Range test					
	3rd day	5th day	7th day	9th day	LSD=25.7
Control	387±44.51	335±80.88	343±84.41	372±46.18	359.4±64.94^{a}
Male	183.4±16.22	156.6±18.33	131.2±23.30	88.8±11.49	140±39.32^{b}
Female	145.8±17.54	114.2±14.82	88.8±9.12	46.4±9.04	98.8±39.22^{c}
LSD=29.78	238.73±112.95^{a}	201.93±108.78^{b}	187.66±124.34^{bc}	169.26±152.12^{c}	

***: highly significant; Alphabets (a, b, c and d) are represented by software for statistical difference among the mean value of different group.

The CAT activity in DF patients was measured from 3rd to 9th day of infection (table 9). The production of hydrogen peroxide was very high during dengue infection. The CAT level was changed in a time-dependent manner. However, the level showed no significant difference on 7th and 9th day of fever. The CAT level was 4.52±0.82 μmol/mol of protein for control, and gradually decreased from 3rd day (3.38±1.17 μmol/mol of protein) to 9th day (2.04±1.88 μmol/mol of protein) of infection. The result also showed a statistical difference in CAT levels between males (2.3±1.36 μmol/mol of protein) and females (1.2±0.78 μmol/mol of protein). The decrease in the CAT level is caused by hyper-production of ROS. It is suggested that decrease in the CAT is caused by imbalance of total antioxidative system in DF.

Table 9 Effect of DF duration on CAT levels

Source	Sum of square	Degree of freedom	Mean Square	Frequency	*P*-value
Sex (S)	85.81	2	42.90	75.39	0.000***
Duration (D)	16.03	3	5.34	9.38	0.001***
Sex X duration	14.03	6	2.37	4.16	0.001***
Error	27.31	48	0.56		
Total	143.39	59			
Duncan's Multiple Range test					
	3rd day	5th day	7th day	9th day	LSD=0.47
Control	4.16±0.67	4.02±0.74	3.92±0.80	4.52±0.82	4.1±0.73[a]
Male	3.65±1.20	2.81±1.12	1.90±0.89	0.89±0.25	2.3±1.36[b]
Female	2.32±0.81	1.18±0.16	0.84±0.16	0.71±0.43	1.2±0.78[c]
LSD=0.55	3.38±1.17[a]	2.67±1.40[b]	2.22±1.47[bc]	2.04±1.88[c]	

***: highly significant; Alphabets (a, b, c and d) are represented by software for statistical difference among the mean value of different group.

3 DISCUSSION

A total of 101 patients with serotype positive IgM were included in our study. The most common symptoms were fever, headache, myalgia, vomiting and diarrhea. There was no pattern of fever time and peak. The fever ranged from 38°C to 40°C. General haematology characteristics were thrombocytopenia and leucopenia. The platelet count was significantly reduced [(37.38±24.77) $\times10^3$] (*P*=0.000) as compared to controls [(348.8±69.85) $\times10^3$], which was in line with the work of Arshad *et al*[15]. After febrile phase, hydration level of DF patients got better and platelet count was dropped due to hemodilution and/or the entry of colloid. The patients going through hemodilution phase were at a high risk of developing life threatening haemorrhages, such as gastrointestinal bleeding[16]. The platelet counts in this study were also in accordance with those reported by Vaughn *el al*[17].

We found significant increase in hematocrit value and hemoconcentration was documented in 89.3% of our patients as reported in Cuban cases (91.6%)[18]. There is lack of clarity in quantifying the degree and rate of change in hematocrit and platelet count in WHO 2009 criteria[19]. Our findings may provide clinicians with additional quantitative criteria as part of the new WHO warning signs. The decrease in platelet count was associated with change of hematocrit in disease condition causing dehydration and hemorrhage.

In the present study, it was found liver enzymes (ALT/SGOT and AST/SGPT) were directly correlated with liver injury. Dengue also results in acute liver failure which can be diagnosed in patients with features suggestive of dengue: high fever, hemorrhagic manifestations, thrombocytopenia, plasma leak syndrome characterized by increasing hematocrit, gall bladder wall edema etc. Our patients in endemic areas had evidence of dengue infection and developed acute liver failure during the course of this disease while other viral markers were negative. Dengue leads to many disorders including jaundice[20]. In case of skeletal muscle injury, SGOT level was higher than SGPT level, but we found the reverse phenomenon i.e SGOT level was higher than SGPT in DF patients[14, 20, 21].

In the present study, increased levels of ALP and serum bilirubin were found in DF patients, in accordance with those reported by Itha *et al*[14]. The ALT levels in dengue infection tend to be lower than AST levels[21]. We found AST level was significantly higher than ALT, an early indicator of dengue infection. This pattern is similar to that of alcoholic hepatitis and differs from that of viral hepatitis. Excess release of AST from damaged monocytes during dengue infection might be a possible reason[13].

DHF is critically dangerous state of DF and liver injury is a good positive predictive factor for the development of DHF. The severity of hepatic dysfunction was reported to be related with the severity of dengue infection[22].

We thus reported the abnormalities found in liver function test (LFT) and their clinical implications in a large group of patients with DF. Further study has to be made to define the cause of hepatic injury in DF by assessing viral titer, and its correlation with LFTs. Significant values found in total protein test in our study is in line with previous findings[23]. Loss of serum protein might also be related to aberrant coagulation and vascular leakage[24]. Vaughn *et al* have shown increased oxidative stress in DF and its association with disease severity[17].

The liver is a target organ of DF; hepatic involvement ranges from mildly elevated aminotransferases to fulminant hepatic failure leading to death[22]. Pathologically, severe diffuse hepatitis, focal necrosis of hepatic cells and hyaline necrosis of Kupffer cells were found. Liver function tests showed elevated liver enzymes with ALP, AST and ALT, in all days of DF as compared with controls. The ALT levels were increased up to two fold or more in DF patients. The ratio of increase of ALT with controls was 1.45:2.41:2.01:1.79 on 3rd, 5th, 7th and 9th day of DF respectively (table 4), which is in agreement with the data reported by Halstead[25]. The increase in ALT caused the severity of DF. Redox equilibrium plays an important role in maintaining normal function of cells such as activation, maturation, and cell signalling and death[26].

The time-dependent values of platelets were significant. Thrombocytopenia, which developed from onset of fever, appeared on the time points of 3rd, 5th, 7th and 9th day of our study. Thrombocytopenia was common in DF and always found in DHF/DSS[27, 28]. The pathogenesis of thrombocytopenia is weakly understood. It is sug-

gested that thrombocytopenia is a result of depressed platelet synthesis caused by dengue virus-induced bone marrow suppression[29]. One group found that in the presence of virus-specific antibodies the dengue virus of type 2 could bind to human platelets, suggesting that the immune-mediated clearance of platelets was involved in the pathogenesis of thrombocytopenia in DHF/DSS. It has been found that generation of anti-platelet antibodies in a murine model of dengue infection is associated with the transient thrombocytopenia caused by dengue infection[30].

Pathogenic role of anti-platelet autoantibodies during dengue infection is suggested by the cross-reactivity of antibodies directed toward dengue virus proteins, especially NS1, and platelets[31]. Immune-mediated destruction of platelets in dengue infection and the long-term safety of a dengue vaccine could be explained by the production of anti-platelet auto-antibodies whose affinities are enhanced in secondary infection.

In the present study, we evaluated SOD activity and antioxidant capacity in dengue infection and found increase in SOD activity and a low antioxidant capacity and also found the redox status alteration in the sera of DF patients. This finding is in accordance with the study of Schwarz[32], who reported association of increased oxidant and lowered antioxidative serum capacity with viral illness.

Serum samples were collected at three different time points during the acute phase of the disease and the levels of antioxidants were measured for each condition. Both increased and decreased levels of oxidants have been reported in different days. Hence, fluctuation in ROS is a cause or a consequence of the illness[33]. Antioxidant enzyme levels are sensitive to oxidative stress. We found the CAT activity was significantly reduced in DF patients as compared with controls. We also observed reduced SOD activity among patients. Overall, the total antioxidative enzymes were significantly reduced in the DF patients, and decreased level of CAT is used to evaluate the detoxification capacity and is probably related to the high levels of H_2O_2[34]. H_2O_2 is a stable intermediate that can interact with organic metals to generate peroxyl and other ROS[35].

It is possible that during dengue infection, cytokines stimulate leukocytes and endothelial cells, which also contributes to ROS production[36]. Cytotoxic lipid products may modify proteins and cell membranes to induce oxidative stress processes. Loss of membrane function and integrity is a result of process involving ROS attack on polyunsaturated fatty acids and initiation of lipid peroxidation. It has been suggested that the increased vascular permeability observed in DHF is caused by a malfunction rather than a structural destruction of the endothelial cells[37].

The host inflammatory response and disturbance of equilibrium contribute to the pathogenesis of many diseases. In viral infected diseases, pro-inflammatory cytokines might induce oxidative stress. A variety of cellular functions is regulated by GSH, an oxidant present in epithelial cells, by redox-dependent mechanisms. Therefore, viral infection or infection-associated symptoms could be potentially treated by antioxidants.

In this study, we found that the decrease of GSH levels occurred in dengue infection at different days after infection with low DF, indicating a relationship between GSH and viral replication. In the present study, the GSH started decreasing from 3rd day of fever as compared with controls, and lowest GSH level was observed at 9th day of infection. The time dependent data of GSH were significant. The decrease of GSH is in harmony with finding of Seet *et al*[38]. The decrease of GSH level may also contribute to pathogenesis of dengue virus. Our study also suggested that GSH might serve as an alternative strategy for prevention and treatment of dengue infection.

Antioxidants levels were measured in serum samples collected during four different days of DF. Significantly high level of MDA was found in dengue patients, suggesting an increase in ROS generation. Disruption of the redox balance and the dramatic increase of MDA, a byproduct of lipid peroxidation, are consistent with a previous observation[35]. In present study, the highest lipid oxidation was observed at 7th day of DF, which was also reported previously[39]. We observed a decrease in GSH and an increase in SOD activity. This high level of lipid peroxidation may be related to weakened antioxidative defence system, and used to evaluate the detoxification capacity.

In conclusion, findings of present study suggest that in response to viral infection or a consequence of the viral inflammatory process, antioxidant system gets turbulent and DF can serve as an illness model for the metabolic response to an acute, self-limiting tropical viral infection. Hence, our study suggested that ALT, AST, ALP, GSH, CAT and sialic acid levels can be used as reasonable biomarkers for early prediction for severity of viral infection particularly in case of dengue.

Conflict of Interest Statement

Authors declare that there is no conflict of interest.

Authors' contributions

MR, AM, MA, AM and MHQ designed the study and drafted the manuscript. AM, SR, SRK, HMA, RA, and MA performed the experiments. KMK, SQ, BS, and SZ helped to collect the data. AGC, AMA and MHA helped in manuscript writing. SK analyzed the data and critically reviewed the manuscript.

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