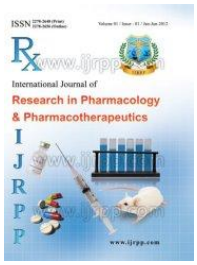
International Journal of Research in Pharmacology & Pharmacotherapeutics



ISSN Print: 2278-2648 ***IJRPP |Vol.4 | Issue 2 | April-June-2015***
ISSN Online: 2278-2656 ***Journal Home page: www.ijrpp.com***

Research article ***Open Access***

Biochemical screening of dengue fever

[1]Shameemunnisa S.A.K and Agnes jenitha X[2]*

[1] PG student, Department of Biochemistry, D.G.G Arts college (W), Mayiladuthurai, 609 001, Tamilnadu, India.

[2]Department of Biochemistry, D.G.G Arts college(W), Mayiladuthurai, 609 001, Tamilnadu, India.

***Corresponding author: X. Agnes jenitha**

E-mail id: agnes_jenita@yahoo.com

ABSTRACT

Dengue virus infection is a serious health problem infecting 2.5 billion people worldwide. Dengue virus belongs to family Flaviviridae, having four serotypes that spread by the bite of infected Aedesmosquitoes. It causes a wide spectrum of illness from mild asymptomatic illness to severe fatal dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS). The relationship of this country with dengue has been long and intense. The first recorded epidemic of clinically dengue like illness occurred at Madras in 1780 and the dengue virus was isolated for the first time almost simultaneously in Japan and Calcutta in 1943–1944. After the first virologically proved epidemic of dengue fever along the East Coast of India in 1963–1964, it spread to allover the country. The first full-blown epidemic of the severe form of the illness, the dengue haemorrhagic fever/ dengue shock syndrome occurred in North India in 1996. Aedesaegyptiis the vector for transmission of the disease. Vaccines or antiviral drugs are not available for dengue viruses; the only effective way to prevent epidemic dengue fever/dengue haemorrhagic fever (DF/DHF) is to control the mosquito vector, Aedesaegypti and prevent its bite. This country has few virus laboratories and some of them have done excellent work in the area of molecular epidemiology, immunopathology and vaccine development. Selected work done in this country on the problems of dengue is presented here. Dengue fever is a re-emergent and challenging public health problem in the world. In this review we will give an overview of the infectious DENV and will discuss the viral and host factors that are important in controlling DENV infection.

Key words: Dengue fever, Viral infection, Biochemical screening.

INTRODUCTION

Dengue fever is an infectious disease carried by mosquitoes and caused by any of four related dengue viruses. This disease used to be called "break-bone" fever because it sometimes causes severe joint and muscle pain that feels like bones are breaking. Health experts have known about dengue fever for more than 200 years (1).

Dengue is regarded as one of the most important arboviral infections in the world. Dengue fever (DF), including its variants, dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS), is caused by four antigenically distinct but related dengue viruses (DENV-1, DENV-2, DENV-3 and DENV-4),also known as serotypes, belonging to genus Flavivirus, family Flaviviridae(2).

DV is a positive-stranded encapsulated RNA virus and is composed of three structural protein genes, which encode the nucleocapsid or core (C) protein, a membrane-associated (M) protein, an enveloped (E) glycoprotein and seven non-structural (NS) proteins. It is transmitted mainly by Aedes aegypti mosquito and also by Ae. albopictus. All four serotypes can cause the full spectrum of disease from a subclinical infection to a mild self-limiting disease, the dengue fever (DF) and a severe disease that may be fatal, the dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS). The WHO 2009 classification divides dengue fever into two groups: uncomplicated and severe, though the 1997 WHO classification is still widely used. The 1997 classification divided dengue into undifferentiated fever, dengue fever (DF), and dengue haemorrhagic fever (DHF) (3).

Dengue virus infection may manifest clinically as undifferentiated fever, dengue fever (DF), dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS). Renal injury comprising creatinine increase, proteinuria, glomerulonephritis, acute kidney injury (AKI) and haemolytic uraemic syndrome has been reported in dengue patients (4).

MATERIALS AND METHODS

The present study was carried out in Govt. Hospital at Mayiladuthurai, 25 patients were selected for the study. The blood samples were collected from the patients and biochemical analysis were done.

Group I : Indicates Normal individuals

Group II : Indicates Dengue patients

- To determine the Haemoglobin, PCV, Platelets, PT
- To estimate the Serum creatinine, Serum bilirubin, SGOT, SGPT
- To screen the LDH, Serum lactate, INR
- To estimate the Serum IgG and IgM Antibodies.

1. Estimation of hemoglobin

Cyanmethamoglobin method by Dacie, 1968

20 µl of blood was added to 2ml of Drabkin solution. Mixed well and allowed to stand for 10 minutes. Read the absorbance at 540nm with values was expressed as g/dl. Drabkin solution as blank. Read the absorbance of the standard in the same way.

2. **Packed cell volume**

This method was done by karmen et al., 1955

Oxalated blood was taken and mixed thoroughly by repeated inversion and bill in klintrobes tube up to mark. Centrifuged at 2500 r.p.m for 30 minutes. The original column of blood in the tube was 100 mm. The volume of packed cells could be read directly as a percentage.

3. Estimation of platelets

Direct method by Maspesv, 1955

Aspirated a little of platelet diluting fluid in to the RBC pipette and expel the fluid. Make a dilution of blood 1 in 200 with diluting fluid as described in RBC count. Keep the counting chamber in a petridish containing wet cotton wool. Wait for 10 minutes, until the platelets have settled. Count all the platelets in the whole finely ruled area red cell using high power objective. Platelets are lilac –coloured, 1/7 to ½ the diameter of RBC and usually oval, rod, or comma – shaped.

4. Estimation of PT (Prothrombin time)

This method was done by Biggs, 1962.

The reagent vial was maintained at room temperature (20 -30° C).Mixed the content of the vial to homogenise the suspension completely. Aspirated from the reagent vial enough reagent for immediate testing requirement in a thoroughly clean and dry test tube. (Plastic test tubes are preferred). Prewarm the reagent and brought to 37°C before use in test procedure (5 - 10 minutes may be required depending on the reagent volume to attain 37°C before testing). Recap the reagent vial and replace immediately to 2 - 8 °C. 0.1ml of plasma was added into the tube and places the tube in a water bath for 3 to 5 minutes at 37°C. The tube forcibly added 0.2ml of UNIPLASTIN reagent (prewarmed at 37°C for atleast 3 minutes) and simultaneously start a stop watch. Shake the tube gently to mixed contents. Gently till the tube back and forth and stop the stop watch as soon as the first fibrin strand was visible and the gel/clot formation begins. Recorded the time in seconds. Repeat steps 4 -6 for a duplicate test on the same sample. The average of the duplicate test values. This was prothrombin time (PT).

5. Estimation of serum creatinine

Jaffe's method by Folin, 1991

0.2ml and 0.4ml of working standard solution were pipetted out into two test tubes. 0.1ml of serum was taken as the test. The solution in the tubes were made upto 2ml of water with blank and added 1ml of picric acid reagent and 1ml with NaoH reagent. Then allowed to stand for 30minutes.Then the colour was developed at measured 500nm.

6. Estimation of bilirubin

Malloyevetly method by Malloy et al., 1937

Two test tubes were taken and to placed 0.2 ml of serum and 1.8 ml of distilled water. To test added 0.5 ml of diazoreagent and to the blank added 0.5 ml of 15% hydrochloric acid, added 2.5 ml of methanol and allowed it 10 stand for 30 minutes. The colour developed was read at 340 nm For standard curse, pipette out 0.2 to 1.0 ml of working standard and the volume was made upto 9 of direct bilirubin can be determined.

7. Serum glutamate oxalo-acetate transaminase

2,4- Dinitrophenyl hydrazine method of King (1937)

To the test" tube, 1 ml of buffer substrate and 0.2 ml of serum was added, into the test tube. The control 1 ml of buffer substrate was taken. Both the tubes were incubated at 37°c for 1 hour. After incubation period 0.2 ml of serum was added to the control. Standard was prepared by added 0.1-0.5 ml of the standard pyruvate and made up to 1 ml with buffer. 1 ml of the phosphate buffer was taken blank. Two drops of aniline citrate reagent were added to all the tubes. Mixed and added 0 .1 ml of 2,4- di nitrophenyl hydrazine reagent and incubated test tubes at 37°c for 20 minutes. Then 10 ml of 0.4N sodium hydroxide was added and incubated at 37°c for 10 minutes the brown colour was developed and read at 530 nm.

8. Estimation of serum glutamate pyruvate transaminase

2, 4- Dinitrophenyl hydrazine method of king (1937).

1ml of substrate was pipetted out in to 2 test tubes. The tubes were kept in boiling water bath for few minutes at 37°c to on one tube o.2 ml of serum was added standard was produced by talking (0.1-0.5 ml) of the standard pyruvate and made upto 1 ml with phosphate buffer. 1 ml of phosphate buffer was taken as blank. To all the tubes two drops of aniline citrate reagent was added, mixed it followed by the addition of 2,4- dinitrophenyl hydrazine. Incubated the tubes at 37°c for 20 minutes. Then 10 ml of 0.4 N sodium hydroxide was added and incubated at 37°c for 10 minutes. The brown colour developed was read at 520nm.

9. Estimation of LDH

This method was done by Wootton, 1974

1ml of buffered substrate and 0.1ml of serum were taken and mixed well. Place the mixture in water bath at 25±0.2°C. After a few minutes, started the reaction by added 0.1ml of NADH solution. Incubated for exactly 15 minutes, remove the tube from the bath and immediately added 1ml of dinitrophenyl hydrazine solution with mixing.

10. Serum lactate

This method was done by Wilkinson, 1962. Pipetted 1.40ml reduced NAD solution into a cuvette. 0.05ml serum was mixed and placed in the rack. Insert into the reaction rate analyser that has previously been set to dispense 0.10ml sodium pyruvate. Set background compensation to a perture 0.3 and measuring range with full scale deflection 0.2. Use a measuring time of one minute and chart speed 50mm/minute. Measure the rate of change of optical density.

11. INR

International normalized ratio by colman, 1994.

Reagent vial was maintained in room temperature (20 – 300 C).Mixed the contents of the vial to homoggenise the suspension completely. Aspirated from the reagent vial enough reagent for immediate testing requirement in a thoroughly clean and dry test tube. (plastic test tubes are preferred).Prewarm the reagent and bring to 37°C before use in test procedure (5 - 10 minutes may be required depending on the reagent volume to attain 37°C before testing). Recap the reagent vial and replace immediately to 2 -

8 °C.To a tube added 0.1ml of plasma (ppp) and place the tube in a water bath for 3 to 5 minutes at 37°C.To the tube forcibly added 0.2ml of UNIPLASTIN reagent (pre warmed at 37°C for at least 3 minutes) and simultaneously start a stop watch. Shake the tube gently to mixed contents. Gently till the tube back and forth and stop the stop watch as soon as the first fibrin strand is visible and the gel/clot formation begins. Record the time in seconds. Repeat steps 4 -6 for a duplicate test on the same sample. Find the average of the duplicate test values.

12. Serum IgG & IgM antibodies (Antigen & Antibody Combi Test) (1965)

Open the cassete. It attached two cassettes.

NS1 Cassete in sample then other cassete blanks to antibodies IgG&IgM.

100µl serum was added in NS1 cassete. Pink colour band was produced in the space in mentioned positive. And then serum and 2drops of buffer solution were added in second cassete. IgG and IgM band were produced in all the space NS1 as served as control.

RESULTS AND DISCUSSION

Dengue is the most common arthropod-borne viral infection in the world. The disease is endemic in more than 100 countries throughout Africa, the Americas, the Eastern Mediterranean, South-East Asia, and the Western Pacific. There are four distinct serotypes of dengue virus (DENV) and each of these serotypes can cause disease symptoms ranging from self-limited febrile illness called dengue fever (DF) to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Infection with one serotype confers protective immunity against that serotype but not against other serotypes (5).

Dengue Fever (DF) is now endemic in more than 100 countries and 100 million cases of DF and half a million cases of dengue hemorrhagic fever (DHF) occurs annually in the world. Tropical countries are most seriously affected by DF as environmental conditions of the tropics favor the development and proliferation of Aedes Aegypti, the principal vector (6).

In our present study, we have selected 25 patients suffering from dengue fever in and around Mayiladuthurai. The serum samples are collected from Government hospital, Mayiladuthurai. The analysis of serum has been done in laboratory and results are discussed. Serum has also been taken from normal subjects when compared to dengue patients.

Group I: Indicates normal individuals.

Group II: Indicates dengue patients.

Table: 1 CONTENT OF BIOCHEMICAL PARAMETERS IN GROUP I AND GROUP II

S.NO	PARAMETERS	GROUP I	GROUP II
1.	Hemoglobin	11.5 – 15.5 gms%	5.4gms%
2.	PCV	36 – 52 %	29.2%
3.	Platelets	1.5 – 4.5 lakhs/cu mm	0.85 lakhs/cu mm
4.	PT	14±2 seconds	21 seconds
5.	Serum Creatinine	0.5 – 1.1 mg/dl	1.92 mg/dl
6.	Serum Bilirubin	0.3 – 1.2 mg/dl	1.24 mg/dl
7.	SGOT	Upto 40 IU/L	65.3 IU/L
8.	SGPT	Upto 40 IU/L	72.4 IU/L
9.	LDH	160 – 320 U/L	563 U/L
10.	Serum Lactate	0.5 – 2.2 mmol/L	2.75 mmol/L
11.	INR	0.5 – 1.1 seconds	2.11 seconds
12.	Serum IgG&IgM antibodies	Nagative	Positive

The table 1 represents the normal values of biochemical parameters such as Hemoglobin, PCV, Platelets, PT, Serum Creatinine, Serum Bilirubin, SGOT, SGPT,LDH, Serum Lactate, INR, Serum IgG

& IgM Antibodies in clinically healthy individuals (Group I) when compared to dengue patients (Group II).

The mean difference between haematological values obtained from dengue patients were compared with those non-dengue individuals

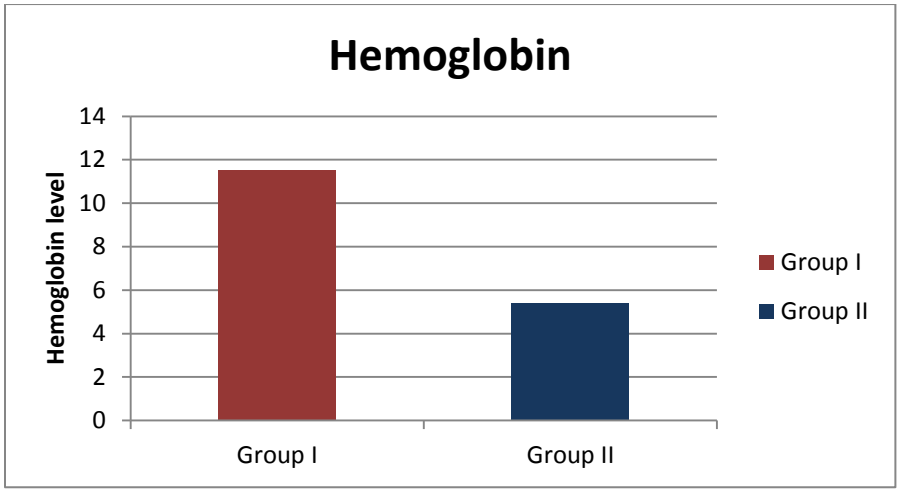


Figure : 1 CONTENT OF HEMOGLOBIN

Fig 1 shows that the level of Hb is decreased in dengue patients (5.4 gms %) when compared to normal healthy individuals (11.5 – 15.5 gms %).

In dengue patietns Hb levels are low as compared to Healthy control group where as platelets levels show a decrease in Dengue patients but sometimes it may also be increased due to other bacterial infections. Reduction of several blood parameters such as WBC, platelets, Hb and percentage of neutrophils below the normal range was more prominent in RT- PCR positive samples of dengue compared to that of RT-PCR negative samples of dengue. (7).

The Hb level in the Healthy Controls is 10.6 – 15.0 gm/dl and the mean value is 12.49 and S.D value is ± 1.13. In dengue patients chronic range 5.4 – 12.7 g/dl and mean value is 8.79 (8).

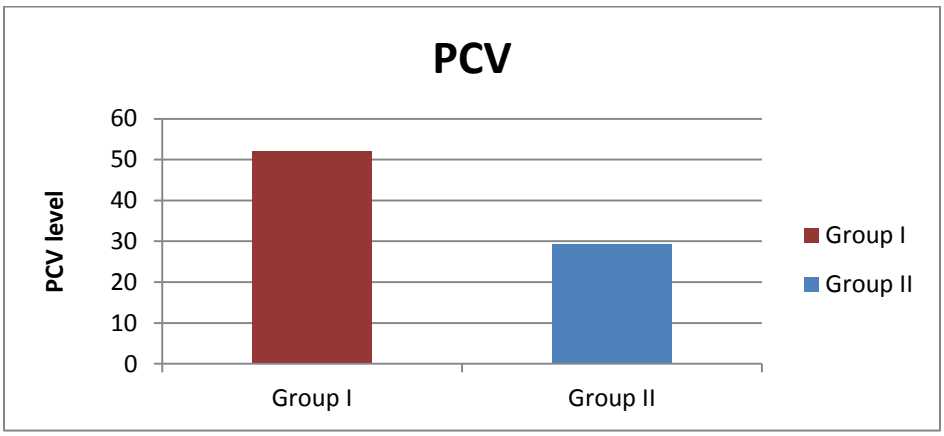


Figure : 2 CONTENT OF PCV

Fig 2 indicates the level of PCV is decreased in dengue patients (29.2%) when compared to normal healthy individuals (36 – 52%).

The hematocrit (Ht or HCT, British English spelling haematocrit), also known as packed cell volume (PCV) or erythrocyte volume fraction (EVF), is the volume percentage (%) of red blood cells in blood. The packed cell volume (PCV) can be determined by centrifuging heparinized blood in a capillary tube

(also known as a microhematocrit tube) at 10,000 RPM for five minutes. This separates the blood into layers. The volume of packed red blood cells divided by the total volume of the blood sample gives the PCV. Since a tube is used, this can be calculated by measuring the lengths of the layers (9).

Elevation of several blood parameters such as ALT, AST, PCV and percentage lymphocytes (however, total lymphocyte count was lower than that of RT-PCR negative group, as the total WBC count was lower) above the normal range was more prevalent in RT-PCR positive samples of Dengue compared to that of RT-PCR negative samples of Dengue(10).

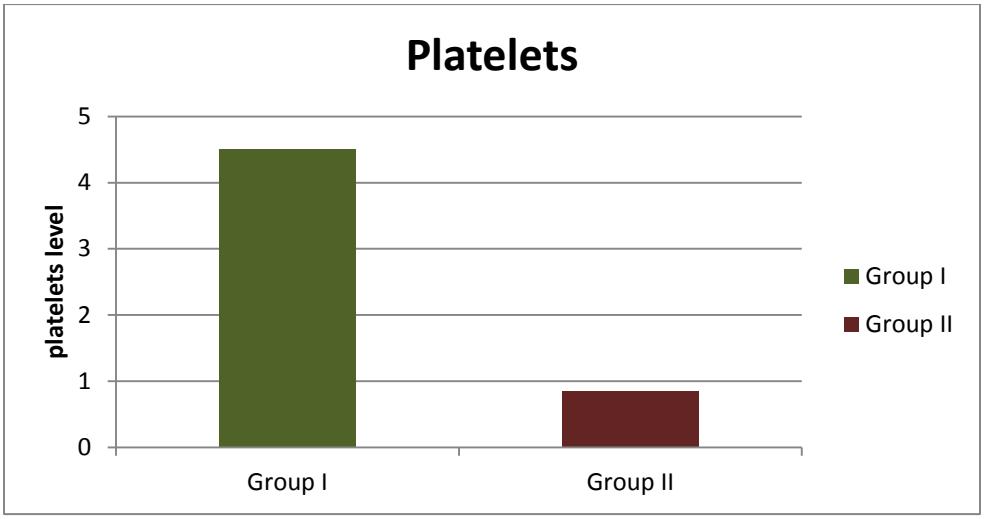


Figure : 3 CONTENT OF PLATELETS

Fig 3 shows that the level of platelets are highly decreased in dengue patients (0.85 lacks/ cu mm) when compared to normal healthy individuals (1.5 – 4.5 lacks/cu mm).

The study also depicted that platelet count is comparatively low among dengue patients i.e 20,000-80,000 cells/cumm as compared to Healthy control groups in which platelet count ranges from 1,71,000-3,12,000 cells/cumm. Dengue hemorrhagic fever was labeled in patients with recent fever, hemorrhagic tendencies, thrombocytopenia with platelet counts below 100,000/mm, and hematocrit 20% above average for age, as per the World Health Organization (WHO) criteria (11).

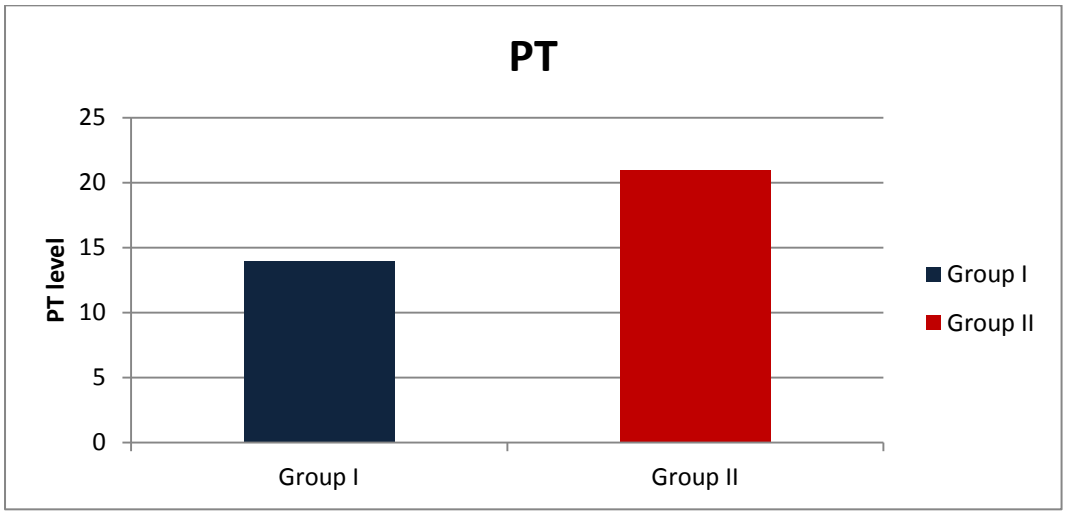


Figure : 4 Content of prothrombin time (pt)

Fig4 indicates the Prothrombin time is significantly elevated in dengue patients (21seconds), when compared to healthy control group individuals (14±2seconds).

The control range of Prothrombin time is 12 -16 sec and mean value is 13.65, its S.D value is ± 1.68.The chronic value is 14 -42 sec, mean value is 26.4 and S.D value is ± 9.57. PT values are increased in dengue patients because the blood clotting mechanism is affected severly (12).

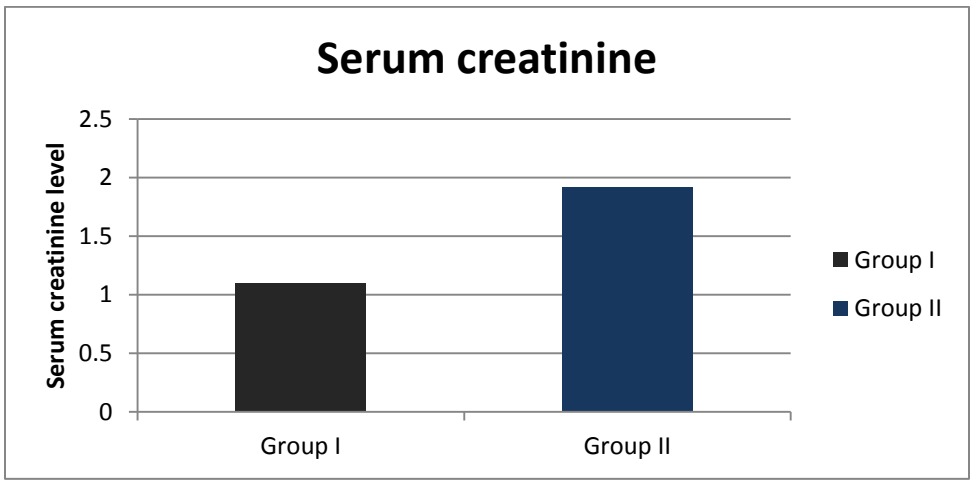


Figure : 5: CONTENT OF SERUM CREATININE

Fig 5 shows that the level of serum creatinine increased in dengue patients (1.92mg/dl), when compared to normal healthy individuals (0.5 – 1.1mg/dl).

Dengue Fever (DF) is only rarely considered as a cause of acute liver failure even globally and only a few case reports of acute hepatic failure and encephalopathy occurring in DF in adults are available. Report a case of Acute Liver Failure due to Dengue during a major outbreak in 2010 in Chitwan. (13). Renal insufficiency was defined as increase in serum creatinine. Acute renal failure was defined as oliguria with an increase in serum creatinine (14).

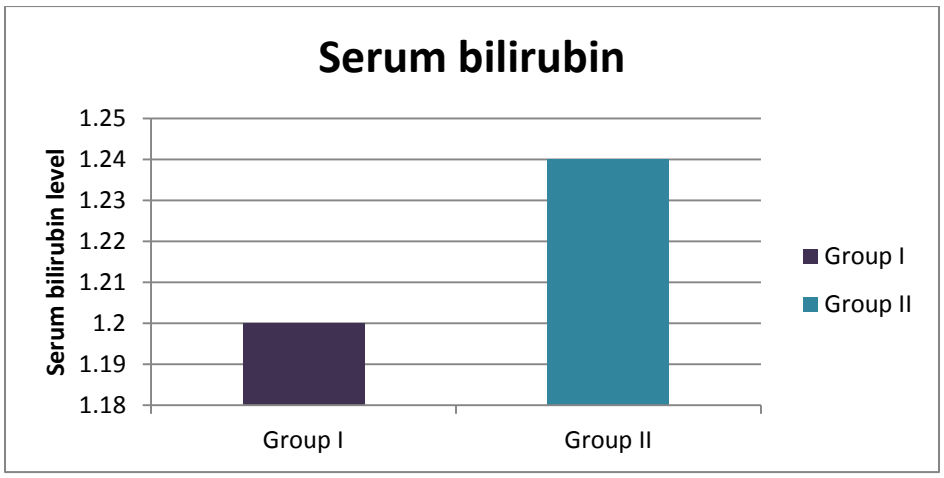


Figure : 6 CONTENT OF SERUM BILIRUBIN

Fig 6 indicates, the serum bilirubin level is increased in dengue patients (1.24mg/dl), when compared to normal persons. The normal range of bilirubin is 0.3 – 1.2 mg/dl. Jaundice is seen infulminant hepatitis

secondary to dengue, but neither of our patients progressed to this stage of hepatic dysfunction (15).

Elevations in total bilirubin and alkaline phosphatase are not common in cholecystitis as the inflammation is restricted to the gall bladder (16).

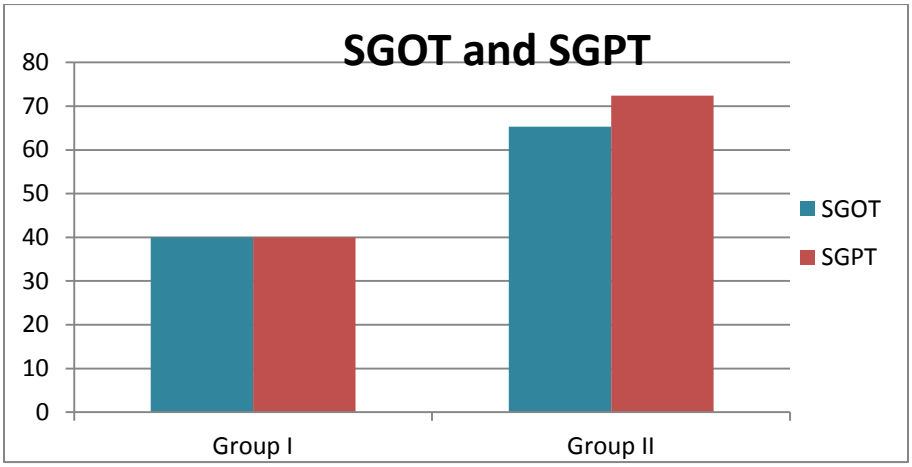


Figure : 7 CONTENT OF SGOT AND SGPT

Fig 7 indicates the, SGOT and SGPT values are raised in dengue patients as compared to healthy controls with SGOT ranging between 74-420 IU/L among dengue patients and 8-40IU/L among healthy control groups. The values of SGPT range from 12-30 IU/L in healthy controls and 53-390 IU/L in dengue patients (17).

Liver enzyme elevation, a common feature in dengue infection was also apparent in our study. AST levels were equal to or greater than those of ALT levels in all of dengue infected patients, a finding that has also been reported earlier (18).

Levels of AST and ALT in patients with dengue were higher than those in patients with non-dengue febrile conditions. However, these biomarkers were not independent predictors of severity among patients with dengue infection (19).

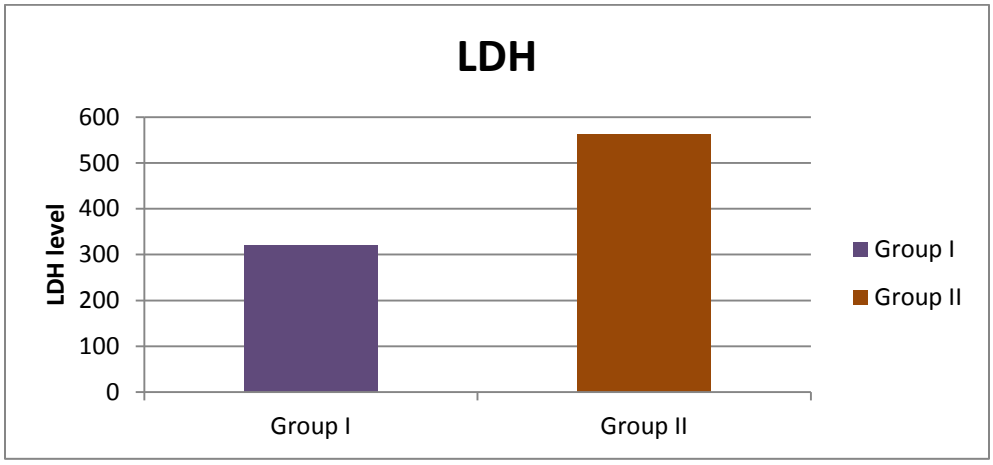


Figure : 8 CONTENT OF LDH

Fig 8 represents the, LDH level is increased in dengue patients (563U/L), when compared to normal persons. The normal level is LDH 160 – 320 U/L.

Moreover, liver damage is a frequent problem in dengue that can also be associated with increased levels of LDH. These biomarkers can predict a more severe form of dengue and could also be indicators of

early tissue injury in the acute phase of dengue infection (20).

Acute hepatic failure, a rarely reported manifestation of dengue hemorrhagic fever, was diagnosed in patients. Deranged liver function in dengue infection can be a result of the direct effect of the virus on liver cells or the unregulated host immune response against the virus. Fulminant hepatic failure occurs because of acute severe hepatitis and massive necrosis of the liver, causing hepatic encephalopathy and even death (21).

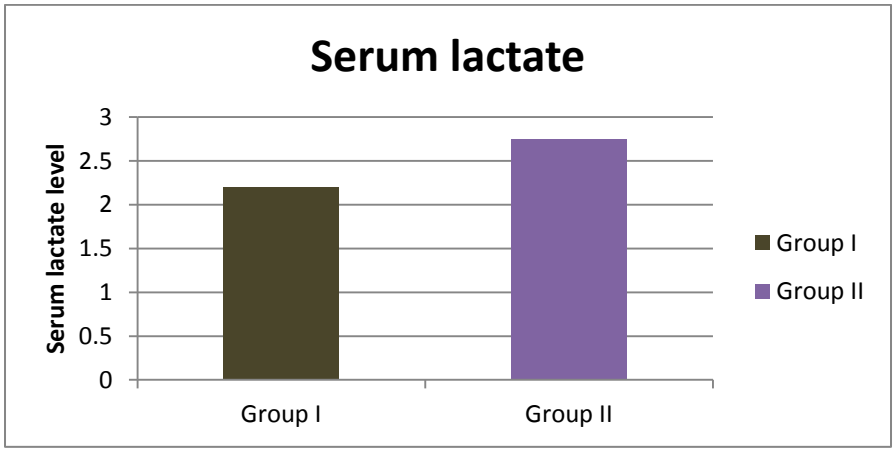


Figure : 9 CONTENT OF SERUM LACTATE

Fig 9 shows that, the serum Lactate level is increased in dengue patients (2.75 mmol/L), when compared to normal persons. The normal range in Serum lactate 0.5 – 2.2 mmol/L.

An increased lactate level represents the increased glycolytic flux due to hyper metabolism; in septic shock, an increased glycolytic flux is due to tissue hypoxia. This suggests that there are two varieties of lactate, namely, the "stress lactate" and the "shock lactate (22).

Blood lactate levels of up to 18 mg/dl (2 mmol/l) are usually defined to be normal for critically ill patients. Hyperl actatemia is defined as lactate levels between 18 and 45 mg/dl without metabolic acidosis whereas lactic acidosis is defined as lactate levels greater than 45 mg/dl and pH below 7.3 (23).

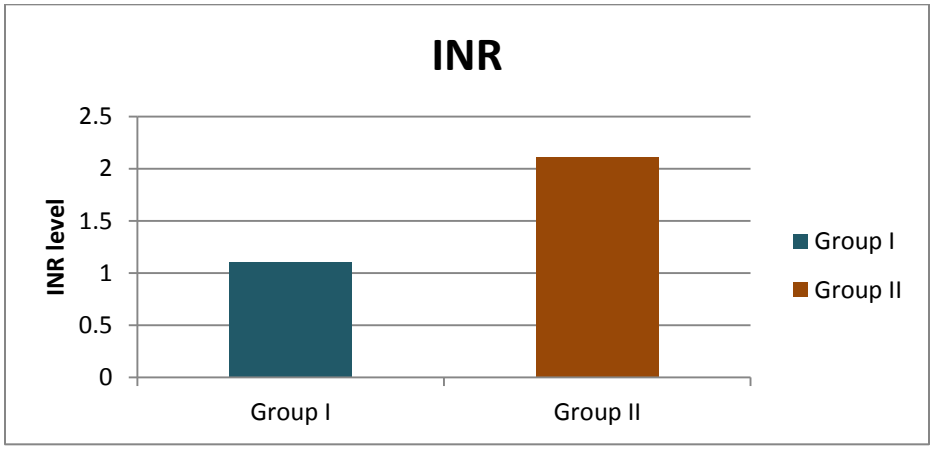


Figure: 10 CONTENT OF INR

International normalized ratio (INR) was only elevated more than 1.5 in Dengue patients. Sonographically, Isolated hepatomagaly observed in 13.72% and 4.94% DF and DHF patients respectively, whereas, ascites and ascites associated with hepatomagaly were observed in 1.64% and

3.29% of DHF patients respectively. The elevation of transaminases is usually less than five-fold greater than upper limit of normal in DF where as in these patients they were more than 10 times higher with an elevated INR (24).

IgG and IgM Antibody card method (combo test)
Plate : 1

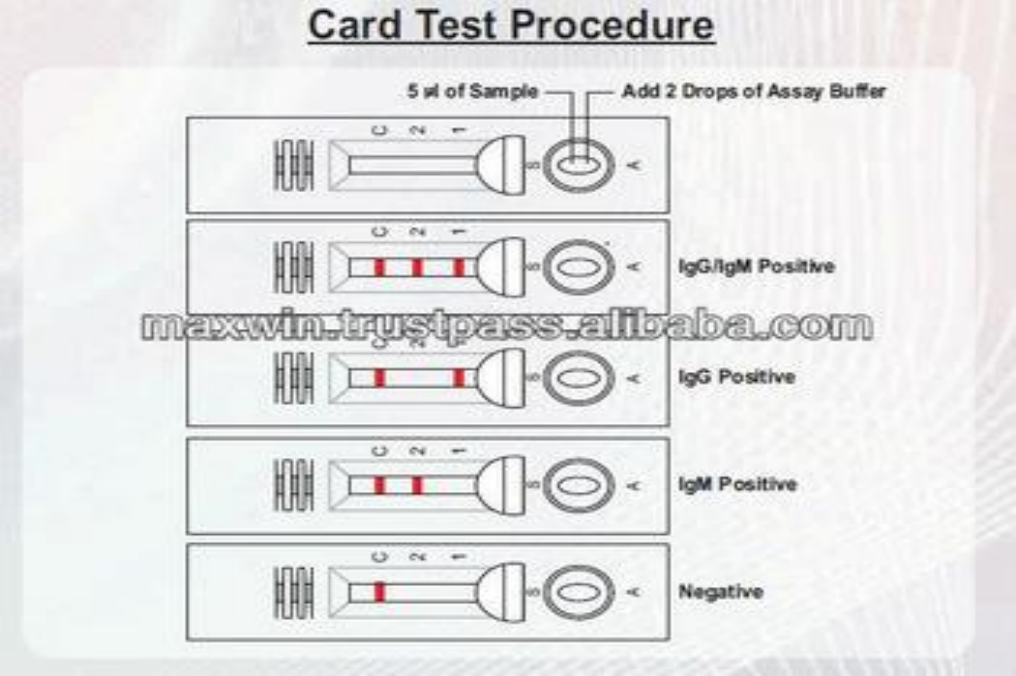


Plate1 indicates, the IgG and IgM antibodies are present in Dengue patients due to band formation.

Serum samples were tested for dengue-specific NS1 antigen and IgM, IgG antibodies. The positivities to only NS1, both NS1 and IgM, and IgM alone and the median duration of fever was five, seven, and ten days, respectively. One case of dengue hemorrhagic fever and one of probable secondary dengue infection with detectable IgG were encountered (25).

The qualitative determination of the antibodies and antigen in the serum samples was done using the Advantage Dengue NS1Ag and AbCombi Card as per the manufacturer's instructions. It is a rapid, solid-phase, immuno chromatographic test, intended for early diagnosis and presumptive differentiation of primary and secondary infections. The test kit has two devices for screening of NS1 antigen and IgM, IgG antibodies. The results were analyzed using percentage, mean, median, and proportion (26).

Antigen was detectable at the earliest on the third day after onset of fever and undetectable beyond seven days. NS1 antigen and IgM antibodies together were positive in 16 cases (27.58%). These patients had an onset of fever five to seven days earlier (median duration of seven days). IgM antibodies were detectable earliest on day five. The remaining seven patients presented with around 10 days of fever (7-10 days) and six (10.34%) were reactive only for IgM antibodies, whereas, one had IgM and IgG antibodies (27).

SUMMARY AND CONCLUSION

It can be summarised of this study that,

In the present study we have selected 25 dengue patients and revealed the haematological and biochemical parameters such as Haemglobin, PCV, Platelets, Prothrombin time, INR, Serum creatinine, Serum bilirubin, SGOT, SGPT, LDH, Serum lactate, Serum IgG and IgM Antibodies.

Hemoglobin (Hb) levels are low as compared to Healthy control group whereas platelets levels show a

decrease in Dengue patients but sometimes it may also be increased due to other bacterial infections.

PCV values shows significantly decreased in dengue patients, when compared to normal subjects. Prothrombin time values show significantly elevated in dengue patients because the blood clotting mechanism is affected severely.

SGOT and SGPT are higher with dengue patients than those with non dengue febrile conditions due to tissue injury of liver. Serum creatinine levels increased in dengue patients due to renal insufficiency. Serum LDH levels is increased in dengue patients when compared to normal individuals due to hepatic failure.

Serum Lactate level is increased in dengue patients represents the increased glycolytic flux due to hyper metabolism. Serum bilirubin and INR levels are elevated in dengue patients due to ascites associated with hepatomegaly. Serum IgG and IgM antibodies are presented in dengue patients. This results indicates positive due to band formation.

It can be concluded of this study that, Dengue disease continues to involve newer areas, newer populations and is increasing in magnitude, epidemic after epidemic. Every aspect of dengue viral infection continuous to be a challenge; the pathogenesis of severe dengue disease is not known, no vaccine is yet available for protection. The best way to prevent dengue viral infection is to take special precautions to avoid being bitten by mosquitoes.

BIBLIOGRAPHY

[1] World Health Organization, Dengue: guidelines for diagnosis, treatment, prevention and control.WHO, 2nd edition, 2009.

[2] Simmons CP, Chau TN, Thuy TT, Tuan NM, Hoang DM, Thien NT, Lien lB, QuyNT, Hieu NT, Hien TT, McElnea C, Young P, Whitehead S, Hung NT, Farrar J (2007) Maternal antibody and viral factors in the pathogenesis of dengue virus in infants. J Infect Dis 196:416–424.

[3] Whitehorn J, Farrar J (2010). "Dengue". Br.Med.Bull. **95**: 161–73. doi:10.1093/bmb/ldq019. PMID 20616106.

[4] SoundravallyR, Sankar P, Hoti SL, Selvaraj N, Bobby Z, Sridhar MG. Oxidative stress induced changes in plasma protein can be a predictor of imminent severe dengue infection. Acta Trop 2008; 106: 156-61.

[5] Anoop M, Issac A, Mathew T, Philip S, Kareem NA, and Unnikrishnan R (2010), Genetic characterization of dengue virus serotypes causing concurrent infection in an outbreak in Ernakulam, Kerala, South India. Indian J.Exp Biol; 48:849-57

[6] Souza LJ, Nogueira RMR, Soares LC, Soares CEC, Ribas BF, Alves FP, et al. The Impact of Dengue on Liver Function as Evaluated by Aminotransferase Levels. Braz J Infect Dis 2007; 11(4): 407-10.

[7] Ghosh K, Gangodkar S, Jain P, Shetty S, Ramjee S, and Poddar P (2008), Imaging the interaction between dengue 2 virus and human platelets using atomic force and electron microscopy; J.Electron Microsc; 57:113-8

[8] Claro, L.B;Tomassini,H.C.and Rosa,M.L;2004.Dengue prevention and control: A review of studies on knowledge, beliefs and practices. Cadernas de saudepublica;20(6):1447-145.

[9] Hedin SG."TheHaematokrit: a New Apparatus for the Investigation of Blood". Skandinavisches Archivfür Physiologie **2**: 134–140,1891.

[10] Gurugama P, Garg P, Perera J, Wijewickrama A, Seneviratne SL: Dengue viral infections. Indian J Dermatol2010; 55(1): 68-78.

[11] da Silvavoorham AM, Dittus WP, Amerasinghe PH, Amerasinghe FP. Serologic evidence for an epizootic dengue virus infecting toque macaques (Macacasinica) at Polonnaruwa, Sri Lanka. Am J Trop Med Hyg. 2009; Feb; 60(2): 300–306.

[12] Coller,B.A.,Clements.D.E., Martyak,T.,Yelmene, Thorne,M. and Parks,D.E;2010.Advances in falvi virus vaccine development. Drugs;13(12):880-4.

[13] Pandey BD, Rai SK, Morita K KuraneI. First case of dengue virus in Techu in Nepal. Nepal Coll. J. 2004Dec; 6(2): 157–159.

[14] Lum LC, Lam SK, George R, Devi S. Fulminant hepatitis in dengue infection.Southeast Asian J Trop Med Public Health 1995;24:467–71.
[15] Roy A, Sarkar D, Chakraborty S, Chaudhuri J, Ghosh P: Profile of hepaticinvolvement by dengue virus in dengue infected children. N Am J Med Sci 2013; 5:480–485.
[16] Bhatty S, Shaikh NA, Fatima M, Sumbhuani AK: Acute acalculouscholecystitis in dengue fever. J Pak Med Assoc 2009; 59:519–521.
[17] Harries,A.D.,Maher,D.and Raviglione,M.C;2001.Report on global surveillence of Epidemic-prone infectious disease.Geneva:WHO;2:69-70.
[18] de Souza LJ, GoncalvesCarneiro H, SoutoFilho JT, Ferreira de Souza T, Azevedo Cortes V, Neto CG, BastosAssed D, Wallace da Silva Siqueira E (2002) Hepatitis in dengue shock syndrome. Braz J Infect Dis 6: 322-327.
[19] Jusuf H, Sudjana P, Djumhana A, Abdurachman SA, 1998. DHFwith complication of acute pancreatitis related hyperglycemia:a case report. Southeast Asian J Trop Med Public Health 29:367–369.
[20] Kalayanarooj S, Vaughn DW, Nimmannitya S, Green S, SuntayakornS, Kunentrasai N, Viramitrachi W, Ratanachu-eke S, KiatpolpojS, Innis BL, Rothman AL, Nisalak A, Ennis FA, 1997.Early clinical and laboratory indicators of acute dengue illness.J Infect Dis 176: 313–321.
[21] Kumar R, Tripathi P, Tripathi S, Kanodia A, Venkatesh V (2008) Prevalence of dengue infection in north Indian children with acute hepatic failure. Ann Hepatol 7: 59-62.
[22] Husain FA, Martin MJ, Mullenix PS, Steele SR, Elliott DC. Serum lactate and base deficit as predictors of mortality and morbidity. Am J Surg. 2003;185:485–91.
[23] Howell MD, Donnino M, Clardy P, Talmor D, Shapiro NI. Occult hypoperfusion and mortality in patients with suspected infection. Intensive Care Med. 2007;33:1892–9.
[24] Nguyen TL, Nguyen TH, Tieu NT, 1997. The impact of denguehemorrhagic fever on liver function. Res Virol 148: 273–277.
[25] Gulati S and Maheshwari A (2007) Atypical manifestations of dengue. Trop Med Int Health 12: 1087-1095.
[26] Franco-Paredes C, Jacob JT, Hidron A, Rodriguez-Morales AJ, Kuhar D, Caliendo AM: Transplantation and tropical infectious diseases. Int J Infect Dis 2010, 14:e189-e196
[27] Wilder-Smith A, Schwartz E. Dengue in travelers. N Engl J Med2004; 353:924–32.