

Case Report

ANALYSIS ON WHOLE BLOOD, SGOT, SGPT, AND TNF- α EXAMINATION IN PATIENTS WITH NON-DENGUE AND POSITIVE DENGUE FEVER (DF/DHF)

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ABSTRACT

Background: In Indonesia has four serotypes, the DEN-1, DEN-2, DEN-3 and DEN-4. The management of Dengue virus becomes difficult because the patients were infected with different clinical profiles depending on the serotypes and genotypes of infecting dengue virus. Consequently, the diagnosis and treatment becomes difficult. *Aim:* The purpose of this study was to identify the difference between the results of laboratory tests between non-dengue fever and positive dengue fever. *Method:* This study was an observational cross-sectional study. Fifteen samples were diagnosed with dengue fever and fifteen samples with negative dengue fever on NSI, IgM / IgG-anti-DHF strip test results. Laboratory tests comprising whole blood, SGOT, SGPT, and TNF- α were first examined when the patient came to the hospital. The collected data were analyzed by Chi-Square test SPSS version 13 for Windows. *Result:* The results of the study in two groups regarding sex, age, days of fever, grade, hemoglobin levels, leukocytes count, platelet count, hematocrit percentage, SGOT levels, and TNF- α level were not significantly different with $p > 0.050$, whereas the SGPT level in non-dengue increased 3 x of normal value of 66.7%, $n = 10/15$ and in positive dengue fever the SGPT level was within normal limits, found in 60%, $n = 9/15$, so there was significant difference with $p = 0.022$ ($p < 0.05$). *Conclusion:* in non-dengue SGOT and SGPT levels increased of 1–3 times the normal value. In positive dengue fever SGOT levels increased 1–3 x normal value, but SGPT levels was within normal value, so SGPT levels can be used as a predictive factor for distinguishing the two types of fever.

Key words: Dengue Fever, Whole Blood, SGOT, SGPT, TNF- α

ABSTRAK

Latar belakang: Di Indonesia memiliki empat serotipe dengue yaitu DEN-1, DEN-2, DEN-3 dan DEN-4. Aspek manajemen virus dengue menjadi sulit, karena orang terinfeksi hadir dengan profil klinis berbeda tergantung pada serotipe dan genotipe dari virus dengue yang menginfeksi, sebagai konsekuensi diagnosis dan perawatan menjadi sulit. *Tujuan:* Tujuan penelitian untuk mengetahui perbedaan hasil pemeriksaan laboratorium antara Demam bukan dengue dan Demam positif dengue. *Metode:* Penelitian ini bersifat observasional dengan bentuk cross sectional. Lima belas sampel didiagnosis demam berdarah dan lima belas sampel negatif demam berdarah dari hasil NSI, IgM/IgG-anti DHF metode Strip. Pemeriksaan laboratorium adalah Darah Lengkap, SGOT, SGPT, dan TNF- α diperiksa saat pertama kali penderita datang ke Rumah Sakit. Data yang terkumpul dianalisis uji Chi-Square dengan SPSS versi 13 for Windows. *Hasil:* Hasil penelitian pada jenis kelamin (sex), Umur, hari demam, Grade, kadar Hemoglobin, jumlah leukosit, jumlah trombosit, persentase hematokrit, kadar SGOT, dan kadar TNF- α pada kedua kelompok tidak berbeda bermakna dengan nilai $P > 0,050$, sedangkan kadar SGPT pada Demam bukan dengue meningkat 3x nilai normal sebesar 66,7%, $n=10/15$ dan Demam positif dengue masih dalam batas normal sebesar 60%, $n=9/15$, sehingga hasil SGPT antara kedua kelompok terdapat perbedaan secara bermakna dengan $p=0,022$ ($P < 0,05$). *Kesimpulan:* Pada Demam bukan dengue kadar SGOT dan SGPT meningkat 1–3x nilai normal. Pada Demam positif dengue kadar SGOT meningkat 1–3x nilai normal, namun kadar SGPT masih dalam batas normal, sehingga kadar SGPT dapat sebagai faktor prediksi untuk membedakan kedua tipe Demam tersebut.

Kata kunci: Demam Dengue, Darah lengkap, SGOT, SGPT, TNF- α .

INTRODUCTION

Dengue fever has become one of the world’s most important diseases caused by arthropods that develop in tropical and subtropical regions. Approximately 100 million cases of dengue infection occur each year, and an estimated 2.5 to 5 billion people worldwide are at the risk of dengue virus infection (Halstead SB, 2002). These four dengue virus serotypes (DEN-1, 2, 3 and 4) can transmit to humans through the bite of female mosquitoes of the genus *Aedes* (Chen LK, et al 2003). Dengue hemorrhagic fever (DHF) are spread throughout Southeast Asia, Western Pacific, and the Caribbean. Indonesia is an endemic region with widespread distribution throughout the country. The incidence of dengue fever in Indonesia between 6 to 15 per 100,000 population (1989–1995), and has risen sharply to 35 per 100,000 population in 1998, while the DHF mortality tends to decline until it reached 2% in 1999 (WHO, 2000).

Dengue fever is an acute febrile illness for 2–7 days, characterized by two or more clinical manifestations such as headache, retro-orbital pain, myalgia/arthralgia, skin rash, petechiae (hemorrhagic manifestations), and leukopenia. The diagnosis of dengue hemorrhagic fever (DHF) can be established when all above are met, and there is at least one hemorrhagic manifestations, such as petechiae, ecchymosis, purpura, epistaxis, gingival bleeding, melena, hemetemesis, thrombocytopenia (< 100.000/uI), and there is minimally one sign of pleural effusion, ascites, or hypoproteinemia, plasma leakage, such as increased hematocrit > 20% compared to a standard age and sex, decreased hematocrit > 20% after fluid therapy than previously hematocrit value (WHO, 1997).

Differences between DHF and DF is the presence or absence of plasma leakage after a phase of fever, and the patient will experience critical phase for 2–3 days. During this phase, the patient has no fever, but at the risk for seizure/shock if not handled with adequate treatment. Continuous abdominal pain is accompanied by vomiting, loss of consciousness, hypotension, restlessness, rapid and weak pulse and hypothermia are the signs and symptoms of dengue shock syndrome. Dengue virus is a non-hepatotropic virus, but it can damage the liver because dengue infection is often unusual and has been described since the 1960s (Burke T, 1968).

According to HY Lei et al. (2001), dengue virus infection affects immune system, such as the change of the CD4/CD8 ratio, cytokines overproduction can infect endothelial cells and hepatocytes with the result of apoptosis and dysfunction of these cells. Coagulation and fibrinolysis systems are also activated during dengue virus infection. Immune response disorder is not only disturbing viral clearing from the body, but cytokines overproduction can affect endothelial cells, monocytes and hepatocytes. Platelet destruction is caused by cross-reaction of anti-platelet autoantibody due to overproduction of IL-6 that plays a major role in the formation of anti-platelet autoantibodies and anti-endothelial cells, and increased levels of tPA and coagulation deficiency.

The level of liver dysfunction in dengue infection varies from mild symptoms, identified through AST and ALT examination, up to severity with jaundice and even fulminant hepatic failure (Seneviratne SL, 2006, Halstead SB, 2002). Liver dysfunction could be a direct effect of viral infection that can be detrimental with consequences of dysregulated immune response to the virus in the host (Seneviratne SL, 2006). However, clinical studies showed that liver involvement in dengue infection, especially in adults, is still rare. With such considerations, the liver function (SGOT and SGPT) examination is worth to be investigated.

MATERIALS AND METHODS

This was an observational cross-sectional study. The sampling process was done at Inpatient Wards, Tropical Infectious Diseases Division, Department of Internal Medicine, Dr. Soetomo Hospital, Faculty of Medicine, Airlangga University, Surabaya from July to December 2011. Inclusion criteria were patients with DF/DHF grade I-IV aged > 15 years, fulfilling the WHO criteria and positive on one of three tests: NS-1 dengue and or IgM/IgG anti-dengue. Patients who showed positive results were classified as dengue positive patients, and patients with a negative result were classified as suffering from non-dengue fever. Patients included in the study were willing to sign informed consent. Sample size was 15 subjects of non-dengue fever and 15 subjects of positive dengue fever.

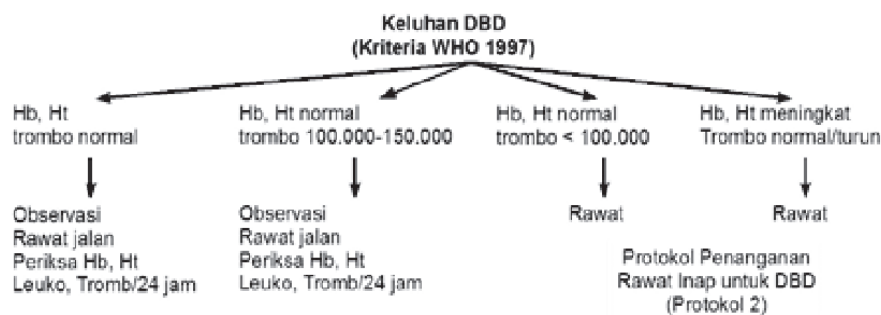


Figure 1. Scheme of DHF symptoms according to 1997 WHO criteria

Research Implementation

Venous blood of the subjects were taken as much as 5 ml, collected in a 2 ml EDTA tubes for whole blood count, and 3 ml was collected in plan tubes to obtain 1 ml serum for SGOT, SGPT, and Tumor Necrosis Factor α (TNF- α). Whole blood count was performed with a Sysmex 4020, while AST and ALT examination was performed with a Hitachi 902. Examination of TNF- α was performed using an ELISA method (Biosource TNF- α EASIA Kit). After all the data collected, the were processed and analyzed using a statistical test (Chi Square test) with SPSS for Window version 13.

RESULTS

1. Sex distribution in groups of non-dengue and positive dengue fever.

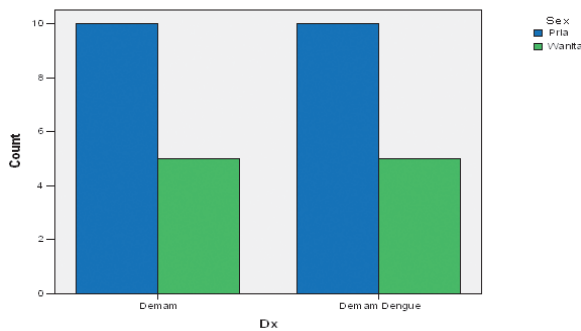


Figure 2. Sex distribution between the groups was not dengue fever and dengue fever positive.

Based on sex distribution, among 30 study subjects in both groups the higher prevalence was found in men (66.7%, n = 10/15). Chi- Square test results showed no significant differences, with P = 0.300 (P > 0.05).

2. Age distribution in groups of non-dengue and positive dengue fever.

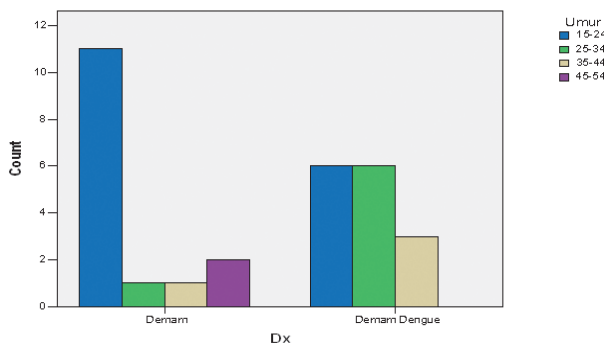


Figure 3. Age distribution between in groups of non-dengue and positive dengue fever.

Based on age distribution, among 30 study subjects, higher prevalence was found between 15–24 years. Chi-Square test results showed no significant differences, with P = 0.128 (P > 0.05).

3. Fever distribution in groups of non-dengue and positive dengue fever.

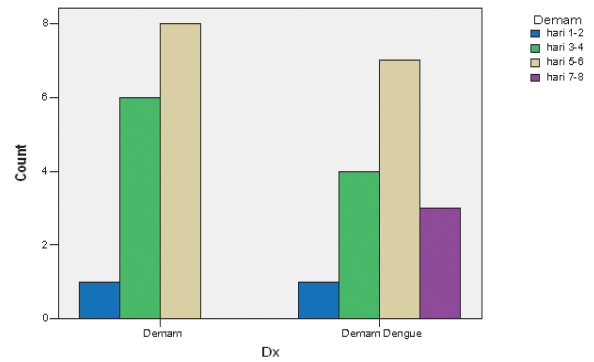


Figure 4. Fever incidence in groups of non-dengue and positive dengue fever.

Based on fever distribution among 30 study subjects, many in both groups come on days 5–6. Chi-Square test results showed no significant correlation, with a value of P = 0.096 (P > 0.050).

4. Grade (disease severity) distribution in groups of non-dengue and positive dengue fever.

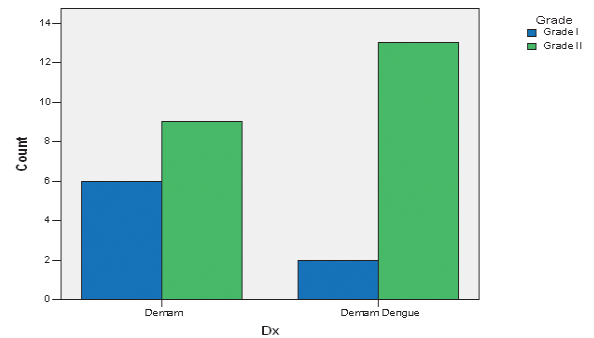


Figure 5. Grade (disease severity) distribution in groups of non-dengue and positive dengue fever.

Based on disease severity among 30 subjects, most visited the hospital in Grade II condition. Chi- Square test results showed no significant relationship with the value of P = 0.090 (P < 0.050).

5. Hemoglobin levels in groups of non-dengue and positive dengue fever.

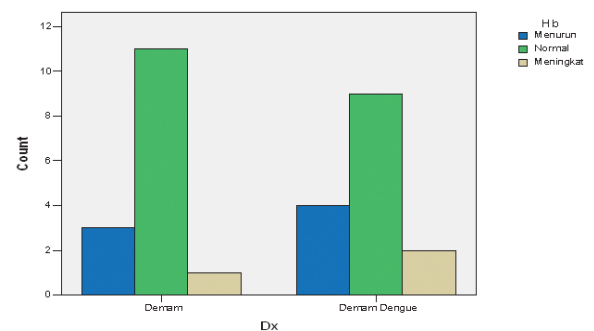


Figure 6. Hemoglobin levels in groups of non-dengue and positive dengue fever.

Based on hemoglobin levels distribution, hemoglobin levels in both groups were still within normal limits. Chi-Square test results showed no significant differences, with $P = 0.434$ ($P > 0.050$).

6. Leukocytes count in groups of non-dengue and positive dengue fever.

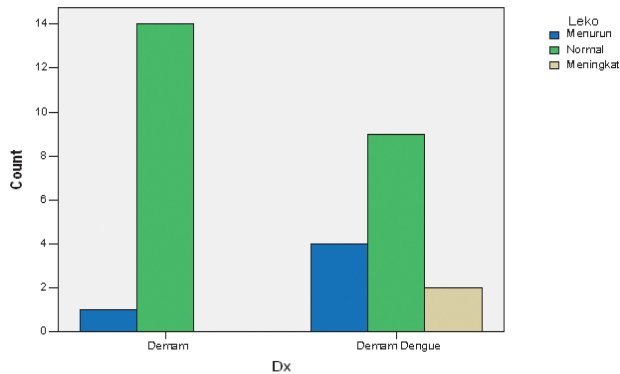


Figure 7. Leukocytes count in groups of non-dengue and positive dengue fever.

The leukocytes count distribution in both groups was still within normal limits. Chi-Square test results showed no significant differences, with $P = 0.274$ ($P < 0.050$).

7. Platelet counts distribution in groups of non-dengue and positive dengue fever.

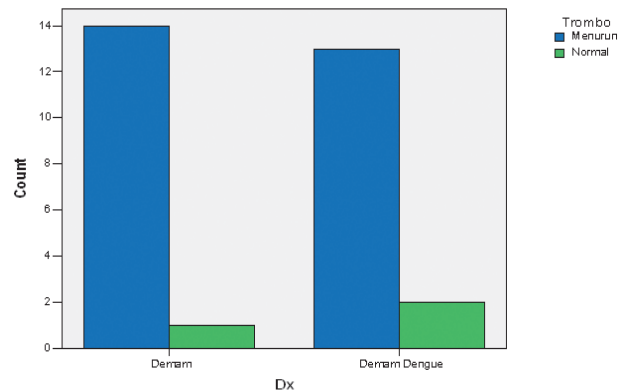


Figure 8. Platelet counts distribution in groups of non-dengue and positive dengue fever.

Based on platelet count distribution, both groups showed reduction less than normal value. Chi-square test

results showed no significant differences, with $P = 0.338$ ($P > 0.050$).

8. Hematocrit percentage in groups of non-dengue and positive dengue fever.

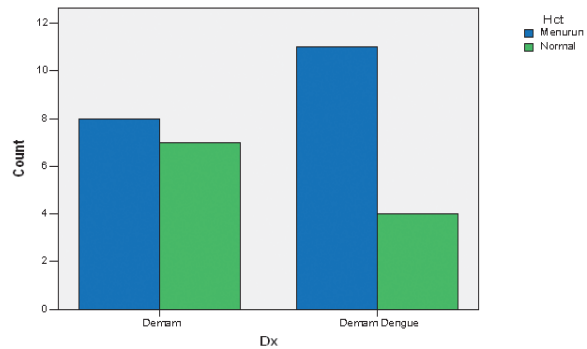


Figure 9. Hematocrit percentage in groups of non-dengue and positive dengue fever.

Based on hematocrit percentage distribution, both groups showed a decrease from normal value. Chi-Square test results showed no significant differences, with $P = 0.161$ ($P > 0.050$).

9. SGOT levels in groups of non-dengue and positive dengue fever.

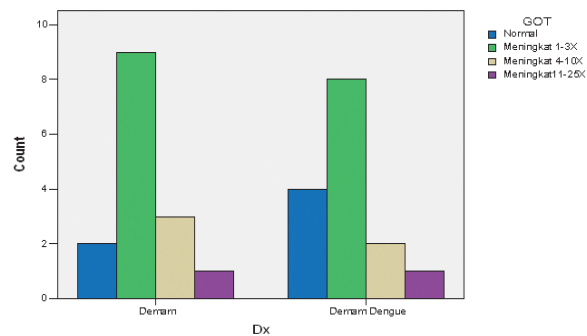


Figure 10. SGOT levels in groups of non-dengue and positive dengue fever.

Based on SGOT levels distribution, both subjects showed increase 1–3 times the normal value. Chi-Square test results showed no significant differences, with $P = 0.143$ ($P > 0.050$).

10. SGPT levels in groups of non-dengue and positive dengue fever.

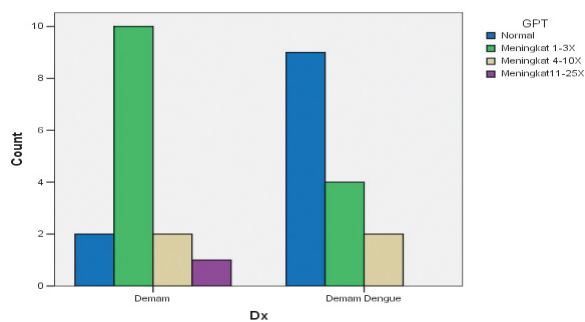


Figure 11. SGPT levels in groups of non-dengue and positive dengue fever.

Based on SGPT level distribution, in non-dengue group the SGPT level increased 1–3 times higher than normal (66.7%, n = 10/15) and in group with positive dengue fever it was still within normal limits (60%, n = 9/15). The results of Chi-Square test showed significant difference with a P value = 0.022 (P < 0.050).

11. TNF- α in groups of non-dengue and positive dengue fever.

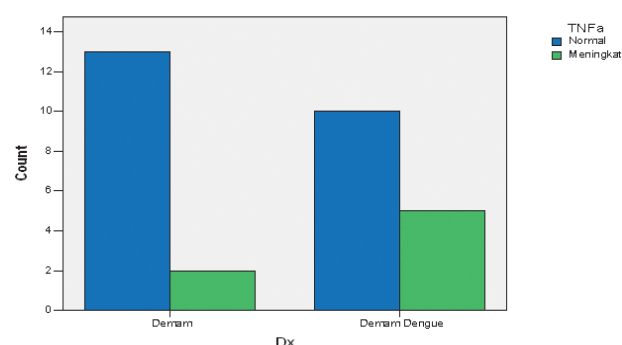


Figure 12. TNF- α in groups of non-dengue and positive dengue fever.

Based on TNF- α distribution, the subjects in both groups were within normal limits. Chi-Square test results showed no significant difference, P = 0.155 (P > 0.050).

Table 1. Laboratory analysis in groups of non-dengue and positive dengue fever on the first day of hospital admission

No.	Variables	Fever N=15	Dengue Fever N = 15	p Value	95% Confidence Interval
1	Sex	Male: 66.7% (10/15)	Male: 66.7% (10/15)	0.300	0.219-4.564
2	Age	15-24 years: 73.3% (11/15)	15-24 years: 40% (6/15)	0.128	-
3	Fever	Day 5-6 53.3% (8/15)	Day 5-6 46.7% (7/15)	0.096	-
4	Grade	Grade II 60% (9/15)	Grade II 86.7% (13/15)	0.090	0.708-26.531
5	Hemoglobin	Normal 73.3% (11/15)	Normal 60% (9/15)	0.248	-
6	Leukocyte	Normal 93.3% (14/15)	Normal 60% (9/15)	0.274	-
7	Thrombocyte	Decrease 93.3% (14/15)	Decrease 86.7% (13/15)	0.388	0.174-26.672
8	Hematocrit	Decrease 53.3% (8/15)	Decrease 73.3% (11/15)	0.161	0.090-1.918
9	SGOT	Increase 1-3x Normal 60% (9/15)	Increase 1-3x Normal 53.3% (8/15)	0.143	-
10	SGPT	Increase 1-3x Normal 66.7% (10/15)	Normal 60% (9/15)	0.022	-
11	TNF- α	Normal 86.7% (13/15)	Normal 66.7% (10/15)	0.155	0.519-20.370
12	Vomiting	Vomiting > 3x 73.3% (11/15)	Vomiting > 3x 53.3% (8/15)	0.161	0.521-11.104

12. Vomiting incidence in groups of non-dengue and positive dengue fever.

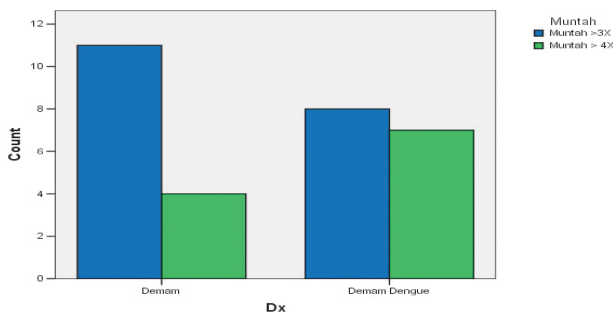


Figure 13. Vomiting incidence in groups of non-dengue and positive dengue fever.

Vomiting incidence in both groups was > 3 times. Chi-Square test results showed no significant differences, with $P = 0.161$ ($P > 0.050$).

DISCUSSION

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are diseases caused by dengue virus infection with clinical manifestations of fever, muscle aches and/or joint pain accompanied with leukopenia, rash, lymphadenopathy, thrombocytopenia and hemorrhagic diathesis. Plasma leakage in DHF occurs characterized by hemoconcentration (increased hematocrit) or the accumulation of fluid in the body cavity. Dengue shock syndrome (DSS) is characterized by dengue shock (Balmaseda A, *et al* 2006, Nasronudin 2007, Soegijanto S., 2006).

NS1 is a glycoprotein with a molecular weight of 50 kDa that is expressed in two forms of membrane-associated (mNS1) and secreted (sNS1) (Paul Y. 2004). NS1 protein has a high immunogenic properties compared with other nonstructural proteins, although its function has not been known. NS3 and NS5 proteins can stimulate humoral immunity, although the effect is very small when compared to the NS1 protein (Earth C, 2006). Upon the entry of dengue virus in the human body, the virus multiplies in the reticuloendothelial cells followed by viremia that lasts for 5-7 days. As a result of this emerging viral infection both humoral immune and cellular responses (antineutralization, antihemagglutination, anticomplementary). The appearing antibodies are generally IgM and IgG. In primary dengue infection, IgM antibodies begin to form, whereas IgG is not yet formed. In secondary infection IgM levels are formed again and IgG antibody levels that already exist will rise (booster effect) (Chen LK, *et al*, 2003, Soegijanto S. 2006).

SGOT, stands for Serum Glutamic Oxaloacetic Transaminase or also called aspartate amino transferase (AST), is an enzyme that is always present in heart and liver cells. AST is released into the blood when the liver or heart is damaged. Very high SGOT levels in blood indicates liver

damage due to viral hepatitis or possible occurrence of heart attacks. Some medications can also raise SGOT level.

SGPT, stands for Serum Glutamic Piruvic Transaminase, or also called ALT (alanine amino transferase), is an enzyme found in the liver cells as well as effective for diagnosing hepatocellular destruction. In small quantities this enzyme found in the heart muscle, kidney and skeletal muscle. Generally, when acute liver parenchymal damage is present, ALT level is higher than AST levels, whereas in chronic process the opposite condition is present.

Reference value of SGPT is 0–40 U/L. Conditions that increase SGPT level > 20 times than normal are usually caused by acute viral hepatitis, liver necrosis (due to drug toxicity or chemicals). SGPT increase of 3–10 times more than normal is caused by mononuclear infection, chronic active hepatitis, biliary extra hepatic obstruction, Reye 's syndrome, and myocardial infarction. SGPT increase of 1–3 times more than normal is caused by pancreatitis, fatty liver, cirrhosis Laennec, and biliary cirrhosis.

SGPT is more specific for liver damage. SGPT is an enzyme produced in the liver cells (hepatocytes), so it is more specific for liver disease compared with other enzymes. SGPT increase usually occurs when there is a damage liver cell membrane. Any type of liver inflammation may cause an increase in liver cell membranes. Inflammation of the liver can be caused by viral hepatitis, some medications, alcohol use, and diseases of the bile duct.

SGOT is a mitochondrial enzyme that is also found in heart, kidneys and brain. So this test is less specific for liver disease. In some cases of liver inflammation, an increase in SGPT and SGOT will be similar (Gowda, Desai, Hull, Math, Kulkarni, Vernekar, 2009). The results of a study in India (Itha S, 2005) revealed that SGPT and bilirubin levels rarely elevated in patients with dengue fever. According to Souza LJ (2002) and Kalayanaraj (1997) SGOT levels in dengue infections tend to be higher than SGPT, but also different from the pattern in patients with viral hepatitis, but similar to that in alcoholic hepatitis. The cause of SGOT > SGPT in dengue patients is excessive SGOT release from monocytes damaged during dengue infection. According to Kuo CH (1992), the determination of SGOT levels increasing 1-3 x than normal value and normal SGPT levels can be an early indicator of dengue virus infection. The findings are similar to the results of this study.

The severity of liver dysfunction in dengue infections is associated with the severity of dengue disease. Therefore, a good predictive factor for DHF severity is to identify the extent of liver damage (Kalayanaraj S, 1997). According to Rajoo Singh Chhina (2008) that the extent of liver damage (SGOT levels) was higher in DF and DHF group, not the SGPT levels, whereas in DSS all liver function tests entirely revealed a rise. Similar data have also been reported by Seneviratne *et al.* (2006) and Souza *et al.* (2004).

The results of this study showed that whole blood count (hemoglobin level, leukocyte count, platelet count, hematocrit percentage, SGOT levels, and TNF- α levels) and

symptoms (fever days, grade, time vomiting) were the same, except in non-dengue fever group the levels of SGPT was 1-3x higher than normal value, whereas in positive dengue fever the SGPT levels was within normal limits.

CONCLUSION

In patients with fever for 4-5 days, which are grouped into non-dengue fever, SGOT levels increased 1-3 x times and SGPT levels increased 1-3 x from normal value. In positive dengue fever SGOT levels increased of 1-3 x, while SGPT level was still within the normal range (< 40 IU/L), so that SGPT levels can be used as a predictive factor for determining positive dengue fever (DF and DHF).

REFERENCES

- Balmaseda A, Hammond SN, Perez L, et.al., 2006. Serotype-specific differences in clinical manifestation of dengue, *Am. J. Trop. Med. Hyg.*; 74 (3), pp. 440–456.
- Burke T, 1968. Dengue haemorrhagic fever: a pathological study. *Trans R Soc Trop Med Hyg.*; 62(5): 682–692.
- Bumi C, Rantam FA., 2006. Determinan virulensi virus dengue dalam Demam Berdarah Dengue. Airlangga University Press. Ed. 2. Surabaya. pp. 239–245.
- Chen Y, Manguire T, Hileman RE, et.al. 1997. Dengue virus infectivity depends on envelope protein binding to target cell heparan sulfat, *Nature*; 3 (8), Article, pp. 866–871.
- Chunlin Z, 2004. Problem encountered in the molecular detection of dengue viruses I dengue diagnostics: proceeding of an international workshop. Geneva Switzerland. WHO. pp. 60–66.
- Clyde K, Kyle JL, Harris E, 2006. Recent advances in deciphering viral and host determinants of dengue virus replication and pathogenesis. *JVI*. 80(23): pp. 11418–11431.
- Halstead SB, 2002. Dengue. *Curr Opin Infect Dis*. 15(5): 471–476.
- Itha S, Kashyap R, Krishnani N, Saraswat VA, Choudhuri G, Aggarwal R. Profile of liver involvement in dengue virus infection. *NatlMed J India*. 2005; 18(3): 127–130.
- Lei HY, Yeh TM, Liu HS, Lin YS, Chen SH, Liu CC., 2001. Immunopathogenesis of dengue virus infection. *J Biomed Sci*. 2001 Sep; 8(5): 377–88.
- Kalayanaraj S, Vaughn DW, Nimmannitya S, Green S, Suntayaorn S, Kunentrasai N, Viramitrachai W, Ratanachu-ek S, Kiatpolpoj S, Innis BL, Rothman AL, Nisalak A, Ennis FA. 1997. Early clinical and laboratory indicators of acute dengue illness. *J Infect Dis.*; 176(2): 313–321.
- Kuo CH, Tai DI, Chang-Chen CS, Lan CK, Chiou SS, Liaw YF, 1992. Liver biochemical tests and dengue fever. *Am J Trop Med Hyg.*; 47(3): 265–270.
- Lum LC, Lam SK, George R, Devi S, 1993. Fulminant hepatitis in dengue infection. *Southeast Asian J Trop Med Public Health.*; 24(3): 467–471.
- Nasronudin, 2007. The prevalence of hypokalemia and hyponatremia in infectious disease hospitalized patients, Dr Soetomo Hospital, Surabaya, 2006 (Tesis). Universitas Airlangga.
- Nguyen TL, Nguyen NT, Tieu NT, 1997. The impact of dengue fever on liver function. *Res Virol*, 148(4): 273–277.
- Seneviratne SL, Malavige GN, deSilva HJ, 2006. Pathogenesis of liver involvement during dengue viral infections. *Trans R Soc Trop Med Hyg*. 100 (8): 608–614.
- Souza LJ, Gonçalves Carnerio H, Souto Filho JT, Souza TF, Cortes VA, Neto CG, Bastos DA, Siqueira EWS, 2002. Hepatitis in dengue shock syndrome. *Braz J Infect Dis.*; 6(6): 322–327.
- Souza LJ, Alves JG, Nogueira RM, Gicovate Neto C, Bastos DA, Siqueira EW, Souto Filho JT, Cezário Tde A, Soares CE, Carneiro Rda C, 2004. Aminotransferase changes and acute hepatitis in patients with dengue fever: analysis of 1,585 cases. *Braz J Infect Dis.*; 8(2): 156–163.
- Soegijanto, S. 2006. Demam Berdarah Dengue edisi kedua. Airlangga University Press. Surabaya.
- Wahid SF, Sansui S, Zawawi MM, Ali RA, 2000. A comparison of the pattern of liver involvement in dengue hemorrhagic fever with classical dengue fever. *Southeast Asian J Trop Med Public Health.*; 31(2): 259–263.
- World Health Organization. Dengue hemorrhagic fever: diagnosis, treatment, prevention, and control, 2nd ed. Geneva: WHO; 1997. p. 1–45.
- World Health Organization: Strengthening the Implementation of the Global Strategy for Dengue Fever/Dengue Hemorrhagic Fever Prevention and Control. Geneva: WHO, 2000