Temperature Effects on Plasma Li-heparin and Transaminase Activity in Children Blood with Tetralogy of Fallot

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Abstract. Tetralogy of Fallot (TOF) is a congenital heart disease that is caused by four heart defects, namely VSD, overriding aorta, obstruction of the right ventricular outflow tract and right ventricle hypertrophy. These abnormalities, which affect the anatomic structure of the heart, cause poor oxygenated blood to be pumped out of the heart. Children have smaller veins than adults, which make obtaining blood sample much harder. An experimental design was utilized, which allows the researchers to manipulate one or more variables and measure the resultant effects on the dependent variables. The population in this study was all vein blood sample of paediatric patients under the age of ten in National Cardiovascular Centre Harapan Kita Jakarta with TOF. Eleven sample were choose randomly as the sample in this study. We took 3 millilitres of blood from each sample and gave Li-heparin. The samples were then stored for 30 minutes at 5º C, 22-25º C and 37 º C. We then used these sample to conduct ALT and AST tests. Based on the results of these tests we conducted descriptive statistical analysis, the normality test, homogeneity test and ANOVA test. We found that there is no significant difference in the results of AST and ALT tests using the three different samples. This study aimed to examine the effects of different temperatures on the volume of Li-heparin plasma and transaminase activity in human blood with Tetralogy of Fallot. The highest plasma volume was obtained from the sample that was put 30 minutes at 37ºC.

Keywords : Tetralogy of Fallot, plasma Li-heparin, Transaminase Activity

1. Introduction

Congenital heart disease is a heart defect that is caused by abnormal formation of the heart during pregnancy. Some of the defects that is commonly found include atrial septal defect (ASD), patent ductus arteriosus (PDA), ventricular septal defect (VSD), transposition of great arteries (TGA), valve defects and Tetralogy of Fallot which is a combination of four heart defect.(1-3)

A routine blood investigation is pivotal in every patient with congenital heart diseases. Haemoglobin and haematocrit might be used as a predictor for hypoxemia. High level of haemoglobin and haematocrit are usually found as the body’s mechanism to compensate with the low level of oxygen in the blood. Patients with congenital heart diseases usually maintain higher levels of haemoglobin and haematocrit in their blood. The haemoglobin level of 16-18 mg/dl and haematocrit level of 50-65% are prevalent in these patients. (4,5)

Tetralogy of Fallot (TOF) is a congenital heart disease that is caused by four heart defects, namely VSD, overriding aorta, obstruction of the right ventricular outflow tract and right ventricle hypertrophy.
hypertrophy. These abnormalities, which affect the anatomic structure of the heart, cause poor oxygenated blood to be pumped out of the heart. This will eventually cause dyspnoea and cyanosis. As previously mentioned, blood investigation is conducted frequently in patients with TOF which most of them are children. Children have smaller veins than adults, which make obtaining blood sample much harder. In addition, high haematocrit level in blood means lower quantity of plasma or serum that can be obtained. Therefore, the blood sample drawn should be used effectively.\textsuperscript{(2,6)}

In conditions where the blood sample is limited such as in conditions with TOF, heparin plasma sample can be used for the blood tests. Heparin plasma sample is preferred because it can be prepared more quickly as the sample does not need to be coagulated.\textsuperscript{(7)} The blood sample drawn can be directly centrifuged and proceed for the prescribed tests. In Certain conditions including TOF, the blood cells increase significantly causing a small volume of plasma in the blood.

Studies revealed that in patients with fever caused by chronic inflammatory diseases, the level of haematocrit dropped causing hemodiluted blood. High temperatures may cause a decrease in haematocrit level. Meanwhile, a refrigerated temperature of 5\(^\circ\) C causes high viscosity blood. Haematocrit level is determined by the number of erythrocytes in the blood. A higher number of red blood cells will cause a higher level of haematocrit and the otherwise. A low temperature may cause morphological changes within the red blood cells, causing an increase in the amount of the erythrocytes. This is known as hemoconcentration of the blood.\textsuperscript{(4,5,8,9)}

Drugs must pass through the liver, to be converted into active forms. Some of these drugs may damage the liver. Take for example acetaminophen, antibiotic, antifungal and most of the cardiac drugs. To evaluate the liver function, blood investigation of aspartate aminotransferase (AST) and alanine transaminase (ALT) may be performed.\textsuperscript{(10)}

AST and ALT tests are usually performed with a serum sample. However, a plasma heparin sample can be used as a promising alternative as it can be prepared more quickly. Hence, the test can be performed earlier. Temperatures has significant effects on enzymes’ activities. The optimal temperature for enzyme to have it greatest catalytic activity is about 25\(^\circ\)C with the human body temperature at around 37\(^\circ\)C. High enough temperature may cause enzymes to denature (break down). Meanwhile, cold temperature causes the inactivity of the enzymes.\textsuperscript{(11,12)}

2. Methods

An experimental design was utilized, which allows the researchers to manipulate one or more variables and measure the resultant effects on the dependent variables. The population in this study was all vein blood sample of paediatric patients under the age of ten in National Cardiovascular Centre Harapan Kita Jakarta with TOF.

Eleven-blood sample were choose randomly as the sample in this study. We took 3 millilitres of blood from each sample and gave Li-heparin. The Li-heparin sample was then put into three tube, with 1 ml each. Haemoglobin levels, hematokrit, AST and ALT were examined using photometric methods (Cobas 6000, negara).

All participants who fulfilled the inclusion criterias were enrolled in this study after their parents or legal guardians giving consent. The characteristics data participant was collected through questionnaires. The laboratory technician was blinded to the assigned drug regimen. Data processing was performed using Statistical Package for Social Sciences for Windows (SPSS) version 24.0, 2016 with \(P\leq0.05\) was significance, and 95\% confidence interval.

3. Results

3.1 Haemoglobin and hematocrit levels in patien with TOF

There were 25 patients with TOF in Harapan Kita National Cardiovascular Centre during period of the study.
Figure 1. Haemoglobin levels in patients with TOF

Figure 1 shows that the haemoglobin level in patients with TOF is maintained above normal range. In this study, the average level of haemoglobin was 19.54 g/dl.

Figure 2. Haematocrit levels in patients with TOF

Figure 2 shows an increase in haematocrit levels, with the average value of haematocrit in this study was 63.12%.
3.2 Effect of temperature on Li-Heparin sample volume

Three tubes of 1 ml Li-heparin sample were kept for 30 minutes at 37°C, 22-25°C and 5°C. Picture 3 shows that the highest increase of Li-heparin volume happened at 37°C.

3.3 Transaminase enzim concetration using heparin plasma at different temperature

The graphic above shows that AST activities using Li-heparin sample were achieved optimally at 37°C. Besides, ALT activities using Li-heparin sample were achieved optimally at 37°C and kept stand for 30 minutes.
4. Discussion
Tetralogy of Fallot (TOF) is a rare congenital heart disease that is caused by four heart defects, namely VSD, overriding aorta, obstruction of the right ventricular outflow tract and right ventricle hypertrophy. These abnormalities, which affect the anatomic structure of the heart, cause poorly oxygenated blood to be pumped out of the heart. This will eventually cause dyspnoea and cyanosis. As previously mentioned, blood investigation is conducted frequently in patients with TOF which most of them are children. Children have smaller veins than adults, which make obtaining blood sample much harder. In addition, high haematocrit level in blood means a lower quantity of plasma or serum that can be obtained. Therefore, the blood sample drawn should be used effectively.

This study examined the effect of varied temperature on the increase of plasma Li-heparin volume. The experiment is carried at 5º C, 22-25º C, and 37ºC. The study found that patients with TOF indeed have an increase in haemtocrit level. All of the sample have haematorict level more than 50% as shown in picture 2 (the normal range of hematocrit level in children is 36 – 40%). Similarly, the haemoglobin levels were found above normal (the normal range for haemoglobin in children is 10 – 16 g/dl). Statistical tests found the average haemoglobin level in this study was 19, 54 g/dl. Meanwhile, the mean of haematocitr level in this study was 63, 12%. These results consistent with the clinical changes in patients with TOF.

This experiment study involved keeping the 1 ml plasma Li-Hepain for 30 minute at different temperatures, namely 5º C, 22-25º C, and 37º C. Each procedure was repeated eleven time and found that the highest increase in the volume of li-heparin plasma happened at 37ºC. The average volume of Li-heparin plasma after being kept at 37º C for 30 minutes was 278, 81 µL. Meanwhile, at the temperature of 22-35º C and 5ºC, the plasma volume increased to 250, 91 µL and 228, 64 µL respectively.

When the sample was put for 30 minutes at 37º C, the mean volume plasma obtained was 36, 60% and increased by 3, 43% compared to the sample put at the 22-25ºC. Meanwhile, the mean volume of plasma Li-heparin obtained at 22-25º C and 5ºC were 33, 17% and 30, 23% respectively. The sample put at 22-25º C had 2, 94% more volume compared to the sample which was put at 5ºC.

This study has proven that the volume of Li-heparin plasma sample can be affected by the given temperatures. The optimal temperature for most enzymes ranges from 0 to 40º C. Very High temperature may cause the enzymes to denature and lost its catalytic functions. Similarly, very low temperature may cause inactivity of most enzymes as the energy for the cellular reactions is insufficient.

The AST tests conducted in this study found that there is a difference in the results by 0, 43 U/L when the sample was put at 5º C compared to 22-25º C. Meanwhile, the difference in the AST activity at 22-25º C compared to 37º C was 0. 39 U/L and the difference in the AST activity at 5º C compared to 37º C was 0, 04 U/L.

The ALT tests conducted in this study found that there is a difference in the results by 0, 05 U/L when the sample was put at 5º C compared to 22-25º C. Meanwhile, the difference in the ALT activity at 22-25º C compared to 37º C was 0. 29 U/L and the difference in the ALT activity at 5º C compared to 37º C was 0, 34 U/L.

As previously mentioned, our study used venous blood sample added with Li-heparin anticoagulant. The samples were then stored for 30 minutes at 5º C, 22-25º C and 37 º C. We then used these sample to conduct ALT and AST tests. Based on the results of these tests we conducted descriptive statistical analysis, the normality test, homogeneity test and ANOVA test. We found that there is no significant difference in the results of AST and ALT tests using the three different samples (sig > 0. 05).

5. Conclusion
This study aimed to examine the effects of different temperatures on the volume of Li-heparin plasma and transaminase activity in human blood with Tetralogy of Fallot. The highest plasma volume was
obtained from the sample that was put 30 minutes at 37°C. ANOVA test of the AST and AST results shows no significant differences in the activity of AST and ALT in all of the samples in this study.

References
[1] A.F. S, R. A, N. M, S. M. Early and late outcomes in repaired tetralloagy of fallot by echocardiography. Ann Pediatr Cardiol. 2014;
[2] Perdhana F, Adriane P. Penanganan Perioperatif Pasien Dengan TOF dan Kardiomiopati Dilatatif Disertai Multiple Thrombus di Semua Ruang Jantung. JAI (Jurnal Anestesiol Indones. 2017;
[3] Sakidjan I. Analisis Kelengkapan Catatan Rekam Medis Kasus Tetralogy of Fallot pada Implementasi INA - CBGS di RSPJN Harapan Kita. J ARSI. 2013;
[4] Oh J, Kang SM, Hong N, Youn JC, Han S, Jeon ES, et al. Hemoconcentration is a good prognostic predictor for clinical outcomes in acute heart failure: Data from the Korean Heart Failure (KorHF) Registry. Int J Cardiol. 2013;
[5] Darawsha W, Chirmicci S, Solomonica A, Wattad M, Kaplan M, Makhoul BF, et al. Discordance Between Hemoconcentration and Clinical Assessment of Decongestion in Acute Heart Failure. J Card Fail. 2016;
[6] Ahmed RA, Salih AF, Mohammed NH. EARLY AND LATE OUTCOMES IN REPAIRED TETRALLOGY OF FALLOT. J Sulaimani Med Coll. 2012;
[7] Gray E, Hogwood J, Mulloy B. The anticoagulant and antithrombotic mechanisms of heparin. Handb Exp Pharmacol. 2012;
[8] Vaduganathan M, Greene SJ, Fonarow GC, Voors AA, Butler J, Gheorghiade M. Hemoconcentration-guided Diuresis in Heart Failure. American Journal of Medicine. 2014.
[9] Brown A, Orav J, Banks PA. Hemoconcentration is an early marker for organ failure and necrotizing pancreatitis. Pancreas. 2000;
[10] Agrawal S, Dhiman RK, Limdi JK. Evaluation of abnormal liver function tests. Postgraduate Medical Journal. 2016.
[11] During EC, All T. Experiment 10 – Enzymes. Laney Coll. 2015;
[12] Daniel RM, Danson MJ, Eisenthal R. The temperature optima of enzymes: A new perspective on an old phenomenon. Trends in Biochemical Sciences. 2001.