JURNAL RISET KESEHATAN
POLTEKKES DEPKES BANDUNG
Vol 16 No 2, Oktober 2024

e-ISSN: 2579-8103
p-ISSN:1979-8253

DIFFERENCES IN THE STABILITY OF CHOLINESTERASE ENZYME ACTIVITY OF SERUM AND HEPARIN PLASMA SAMPLES USING PHOTOMETRIC KINETIC METHOD

Perbedaan Stabilitas Aktivitas Enzim Cholinesterase pada Serum dan Plasma Heparin Menggunakan Metode Kinetik Fotometri

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ABSTRAK

Kesalahan dalam pemeriksaan laboratorium dapat terjadi pada setiap fase proses pengujian, tetapi sebagian besar kesalahan terjadi pada fase pra analitik dengan kontribusi sebesar 70%. Pemeriksaan kolinesterase merupakan pemeriksaan pada keracunan pestisida untuk mendeteksi adanya gangguan kesehatan yang kronis bahkan mematikan. Penelitian ini bertujuan untuk mengetahui stabilitas aktivitas enzim kolinesterase pada sampel serum dan plasma heparin pada suhu ruang. Penelitian ini dilakukan di Laboratorium Kimia Klinik Teknologi Laboratorium Medis Poltekkes Bandung pada bulan Mei 2024. Penelitian ini bersifat quasy experimental dengan memberi perlakuan berupa penyimpanan terhadap sampel serum dan plasma heparin selama 6 dan 24 jam pada suhu ruang yang kemudian aktivitas enzim kolinesterase dibandingkan dengan aktivitas enzim kolinesterase pada serum dan plasma heparin yang segera diperiksa. Pemeriksaan dilakukan dengan metode kinetik fotometrik menggunakan alat fotometer dengan panjang gelombang 405 nm dan dilakukan replikasi sebanyak 3 kali, sehingga didapatkan 90 data yang kemudian diolah menggunakan SPSS dengan uji general linear model (GLM). Secara statistik pada hasil uji GLM pada sampel serum dan plasma heparin menunjukkan nilai p < 0.05 yang berarti terdapat perbedaan stabilitas antara serum dan plasma heparin. Namun, berdasarkan hasil analisa secara klinis nilai Total Error (TE%) dengan penundaan selama 6 jam dan 24 jam terhadap pemeriksaan segera pada sampel serum secara berturut-turut sebesar 1,68% dan 6,44%, sedangkan pada sampel plasma heparin sebesar 3,66% dan 6,95%. Pemeriksaan kolinesterase memiliki nilai TEa% sebesar 8,9%, sehingga baik sampel serum maupun plasma heparin memiliki nilai TE% < TEa%. Berdasarkan hasil tersebut tidak terdapat perbedaan secara klinis.

***Kata kunci**: kolinesterase, plasma heparin, serum, suhu, waktu*

ABSTRACT

Errors in laboratory testing can occur at any phase of the testing process, but most errors occur in the pre-analytical phase with a contribution of 70%. Cholinesterase is an examination in pesticide poisoning to detect chronic and even deadly health problems. This study aimed to determine the stability of cholinesterase enzyme activity in serum and plasma heparin samples. This research was conducted at the Clinical Chemistry Laboratory of Bandung Polytechnic Medical Laboratory Technology in May 2024. This study was quasi-were both of serum and plasma heparin samples stored for 6 and 24 hours ,at room temperature and then the levels were compared were immediately. The examination was carried out by the photometric kinetic method using a photometer with a wavelength of 405 nm and replicated 3 times then the data processed using SPSS with the general linear model (GLM) test. Statistically, the GLM test results on serum and plasma heparin samples showed a sig value. 0.000 where the sig value. <0.05, so there is a difference in stability between serum and plasma heparin. However, based on the

https://doi.org/10.34011/juriskesbdg.v16i2.2777

e-ISSN: 2579-8103
p-ISSN:1979-8253

results of clinical analysis, the Total Error (TE%) value with a delay of 6 hours and 24 hours for immediate examination in serum samples was 1.68% and 6.44%, respectively. While in heparin plasma samples it was 3.66% and 6.95%. Cholinesterase examination had a TEa% value of 8.9%. So the conclusion both serum and heparin plasma samples have TE% < TEa% values, based on these results there is no clinical difference.

Keywords: cholinesterase, plasma heparin, serum, temperature, time

INTRODUCTION

In the healthcare system, disease diagnosis and surveillance rely on laboratory testing. Errors in laboratory testing can occur at any phase of the testing process, but most errors occur in the pre analytical phase which is responsible for 70% of total errors.[1] Specimens for laboratory examination should be examined immediately. However, delays in examination are common in laboratory practice and samples need to be stored. Factors such as equipment malfunction, unavailability of reagents, power failure, distance between the sampling site and the laboratory, too many samples, malfunction of refrigeration equipment and lack of laboratory personnel can cause delays in examination.[2] Storage conditions and temperatures require correct handling. Serum and plasma should ideally be stored at 2-8°C for short-term storage and at -20°C for long-term preservation.[3]

Cholinesterase test is a pesticide poisoning test conducted to identify chronic and even deadly health problems.[4] A decrease in cholinesterase enzyme activity by 30% of normal is one of the signs of pesticide poisoning.[5] According to DGKL (German Society of Clinical Chemistry) the standard method of checking cholinesterase enzyme activity is the photometric kinetic method. This method is simple, accurate, sensitive, and has been adapted to automatic analyzers.[6,7]

Cholinesterase in red blood cells is measured as acetylcholinesterase, while in serum or plasma it is measured as butylcholinesterase. To check for pesticide poisoning, serum and plasma samples are used.[8] This is because butylcholinesterase is commonly used in clinical trials to reflect blood levels of enzymes that chemically inhibit cholinesterase activity and indicate the presence of toxins, such as organophosphate pesticides.[9,10] For examination with plasma samples, heparin and EDTA are some types of anticoagulants that can be used. However, the use of anticoagulants such as oxalate, fluoride, and citrate should be avoided because they can inhibit cholinesterase activity.[11]

Sample stability needs to be known if there is a possibility of examination delays, exhaustion of reagent supplies, or the need for re-examination.[12] Storage and examination delays that occur due to several factors result in sample analysis being delayed for hours to days after collection. Analyte values and enzyme stability in blood are strongly influenced by sample storage time.[2] Therefore, this study vary the storage time for 6 and 24 hours with storage at room temperature to determine whether the samples stored for 24 hours remained stable, both in serum and plasma heparin samples. The most recommended type of heparin to use is lithium heparin, as this form is least likely to interfere with examinations for other ions.[13] The purpose of study was to determine the stability of cholinesterase enzyme activity in heparin serum and plasma samples at room temperature.

METHODS

This study was conducted at the Clinical Chemistry Laboratory of the Poltekkes Kemenkes Bandung, Department of Medical Laboratory Technology with the type of laboratory experimental research and quasy experimental research design. This study was conducted in May 2024 using

https://doi.org/10.34011/juriskesbdg.v16i2.2777

e-ISSN: 2579-8103
p-ISSN:1979-8253

serum and plasma heparin samples from five research units using method of simple random sampling using inclusion criteria of female students related to differences in normal values and hormonal interference, from the Poltekkes Kemenkes Bandung with the inclusion criteria of not menstruating, not taking drugs related to liver function, not being chronically ill. The number of treatments on serum and plasma heparin was 3 treatments each where the samples were examined immediately (0 hours) and stored for 6 and 24 hours at room temperature. Each treatment was repeated 3 times for a total of 90 experimental units.

This study was conducted after obtaining ethical approval from the Health Research Ethics Committee, Health Polytechnic, Ministry of Health Bandung, with number: 51/KEPK/EC/IV/2024. Every action in this study was carried out after obtaining informed consent from the respondents.

Samples were obtained from venous blood using a vacuum needle with one red cap vacuum tube with a volume of 3 mL and one green cap vacuum tube (heparin) with a volume of 3 mL. Next, the blood was centrifuged at a speed of 1500 rpm for 30 minutes. Then the heparinized serum and plasma that were obtained were separated into 3 microtubes of 300 µL each and given a label code. The 1st tube is a sample that is immediately examined, the 2nd tube is stored for 6 hours at room temperature, the 3rd tube is stored for 24 hours at room temperature. Then the cholinesterase enzyme activity examination was carried out immediately as initial data and examined after being stored at room temperature for 6 and 24 hours as an experimental unit.

The data used in this study were primary data obtained from the results of cholinesterase examination on serum and plasma heparin samples examined immediately les than 1 hour and stored for 6 and 24 hours and then measured using the photometric kinetic method. Data were processed using a computer with the IBS SPSS Statistic program to determine whether or not there were differences in cholinesterase enzyme activity between serum and plasma heparin samples with various time delays. Data analysis began with a normality test using the Saphiro-Wilk test. Furthermore, the General Linear Model (GLM) test was conducted to determine the differences between the two groups of data.

RESULT

Serum and plasma heparin samples were examined immediately and delayed for 6 and 24 hours with an average room temperature at the time of specimen storage of 27°C ± 0.56 SD.

Table 1. Samples Storage

Storage Time	Storage Temperature (°C)
Immediately	26,6
6 hours	26.9
12 hours	27.2
18 hours	27.9
24 hours	27.8

The results of the cholinesterase enzyme activity examination in serum and plasma heparin samples were obtained as shown in Tables 2.

Table 2. Cholinesterase Level Enzyme Activity Test in Heparin Plasma and Serum

Samples	Immediately		6 hours		24 hours	
	Heparin Plasma (kU/L)	Serum (kU/L)	Heparin Plasma (kU/L)	Serum (kU/L)	Heparin Plasma (kU/L)	Serum (kU/L)
A	7.32	6.32	7.1	6.25	7.05	5.58
B	9.02	9.37	8.61	9.21	8.18	8.51
C	6.52	6.41	6.14	6.29	6.05	6.14
D	7.72	7.78	7.39	7.62	7.16	7.53
E	9.83	9.83	9.67	9.67	9.15	9.39
Avg	8.01	7.86	7.80	7.66	7.47	7.43
SD	1.33	1.63	1.38	1.60	1.18	1.59

https://doi.org/10.34011/juriskesbdg.v16i2.2777

e-ISSN: 2579-8103
p-ISSN:1979-8253

From tables 2 that samples B and D have higher serum cholinesterase enzyme activity values compared to heparin plasma. While samples A, C and E have higher heparin plasma cholinesterase enzyme activity values than serum its mean that any possibility heparin plasma could interferenece of activity of enzyme CHE. From the table, both heparin plasma and serum samples show a decreasing trend in CHE enzyme activity. Its activity in the serum examined immediately, 6 hours and 24 hours with a percentage decrease of 1.68% and 6.44%, respectively. While in heparin plasma examined immediately, 6 hours and 24 hours with a percentage decrease of 3.66% and 6.95%, respectively. Where the decreasing trend in heparin plasma samples is much higher when compared to the decreasing trend in serum samples.

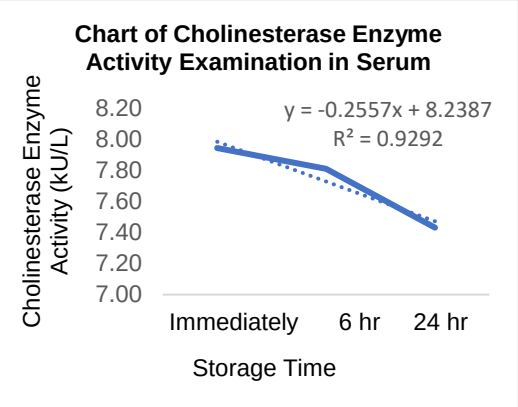

Figure 1. Graph of Cholinesterase Enzyme Activity Test Result in Serum with Storage Time

Based on the results in the graph 1 and 2, it shows that each treatment group has decreased along with the length of time delay. the percentage decrease in cholinesterase enzyme activity in serum samples with a delay of 6 hours and 24 hours against immediate examination is 1.68% and 6.44%, respectively. While in heparin plasma samples, the percentage decrease in cholinesterase enzyme activity with a delay of 6 hours and 24 hours against immediate examination was 3.66% and 6.95%, respectively.

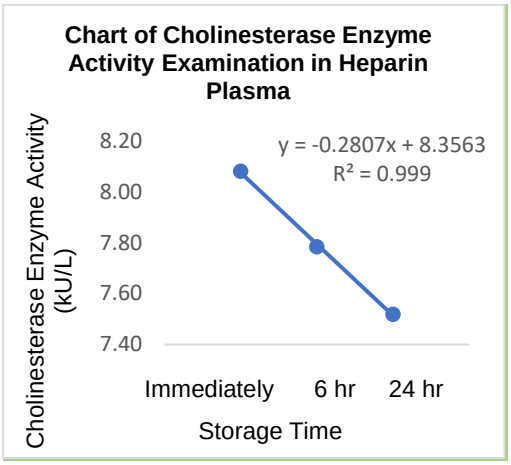

Figure 2. Graph of Cholinesterase Enzyme Activity Test Result in Heparin Plasma with Storage Time

The data were analyzed statistically and clinically by comparing the Total Error (TE) value with the Total Error Allowable (TEa) value. The TEa value for cholinesterase enzyme activity is 8.9%. If the TE value < TEa then there is no clinical difference, but if the TE value > TEa then there is a clinical difference. The results of statistical tests using the general linear model (GLM) are shown in Table 3.

Table 3. General Linear Model (GLM) Test of Cholinesterase Enzyme Activity Between Serum and Plasma Heparin

Source	Factor1	Sig.
Immediately	6 hour	.000
	24 hour	.000

Based on Table 3, it can be seen that there is a significant difference between cholinesterase enzyme activity in serum after 6 and 24 hours storage at room temperature with immediate examination as shown by Sig. values of 0.000 and 0.000, respectively. The total error (TE) value of cholinesterase enzyme activity of serum and heparin plasma examined immediately and delayed at room temperature can be seen in Table 3.

https://doi.org/10.34011/juriskesbdg.v16i2.2777

e-ISSN: 2579-8103
p-ISSN:1979-8253

Table 4. Total Error Values for Serum and Plasma Heparin Samples

Samples	TE% Value		TEa % Value
	6 hr	24 hr	
Serum	1.68	6.44	
Heparin Plasma	3.66	6.95	8.9

Based on the data in Table 4, both serum and plasma heparin samples have TE% < TEa% values, so based on these results there is no clinical difference.

DISCUSSION

In this study, the stability of serum and plasma heparin samples can be affected by temperature and storage duration. Every time the samples were examined, the temperature of the sample storage room was monitored with the available thermometer. The researcher conducted the study in a room with air conditioning (air conditioner) so that the expected temperature at the time of work was 20-25°C, with a temperature bias that is still acceptable in the range ±1°C. This is due to external factors such as many people passing by and extreme weather where in very hot weather conditions have an impact on the room temperature. If the room temperature is unstable and exceeds the standard, the enzyme activity value may decrease.[14] One of the factors that affect the effectiveness of enzyme action is temperature. Inappropriate serum temperature will cause protein denaturation. This will interfere with the active part of the enzyme, lower the effective enzyme concentration and decrease the reaction speed.[15]

After examining cholinesterase enzyme activity in five research units, it was found that the cholinesterase value in serum was higher than the cholinesterase value in heparin plasma. Serum samples have higher enzyme activity because serum does not contain anticoagulants that can change the concentration due to dilution. This is in line with research conducted by Cerón in 2004, where the cholinesterase enzyme activity in serum was higher than the cholinesterase enzyme activity value in heparin plasma.[16]

Figure 2 shows that plasma heparin decreased cholinesterase enzyme activity with the length of time delay. This is confirmed by the R^2 value generated from the graph of 0.999. Thus, there is a correlation between cholinesterase enzyme activity and the time delay of the examination. Statistically, the stability of serum and plasma heparin samples was significantly different. This is in line with Masoon research which states that water structure plays a role in hydrolysis ChE, and suggests that the slow-tending equilibrium between two conformational states of the enzyme, may be related to its regulatory function in dampening the response to certain ligands and irreversible inhibitors.[17]

Statistically, the GLM test aims to see whether or not there is a difference in the average value of each variable tested. Where, in medical inaccuracy value that is usually used is 5%. In this study, the test results showed a statistical difference between cholinesterase enzyme activity in serum samples and heparin plasma samples, with a GLM test Sig. value of <0.05.

The Total Error Allowable (TEa) value between samples that are checked immediately and samples that are delayed can be used to see differences in stability clinically in addition to statistically. There was a decrease in each variation of delay time in both serum and plasma heparin samples which was influenced by storage temperature. This is in line with the percentage decrease in enzyme activity in serum samples stored for 6 and 24 hours at room temperature which were 1.68% and 6.44%, respectively. Meanwhile in heparin plasma samples, the percentage decrease in enzyme activity carried out by storage for 6 and 24 hours at room temperature was 3.66% and 6.95%, respectively.

https://doi.org/10.34011/juriskesbdg.v16i2.2777

e-ISSN: 2579-8103
p-ISSN:1979-8253

According to the Biological Variation (BV), the Total Error Allowable (TEa) value for clinical cholinesterase examination is 8.9%. When viewed from the TEa value, the percentage decrease in cholinesterase enzyme activity in both serum and heparin plasma samples has no clinical difference because the percentage decrease in both is below the TEa value. Thus, both serum and heparin plasma samples can still be used as materials for cholinesterase examination.[18] However, serum samples have a smaller percentage decrease compared to heparin plasma samples. This can be caused by the dilution of the sample when blood is added to anticoagulants. Research conducted by Moorhead et al has examined the negative impact caused by the misuse of heparin anticoagulant where the test results can be falsely low due to unwanted dilution.[19] Thus, results showed that serum samples were more stable than heparin plasma samples. Although the stability of cholinesterase enzyme activity was statistically different, the cholinesterase enzyme activity values remained within the normal range.

CONCLUSION

Statistically there is a difference between the stability of cholinesterase enzyme activity in serum with heparin plasma immediately (less than an hours) and delayed for 6 and 24 hours stored at room temperature clinically there was no difference between the stability of cholinesterase enzyme activity in serum with heparin plasma immediately (0 hours) and delayed for 6 and 24 hours stored at room temperature with TE%<TEa%. Even Although statistically the examination delay factor shows a significant difference, however, with a TEa% value that is smaller than TE%, it can be concluded that delaying the CHE examination is still permitted.

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