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RESEARCH

ANALYSIS OF DECREASED GLUCOSE LEVEL IN STORED SAMPLES CORRELATED TO SERUM SEPARATION AND TEMPERATURE STORAGE

(Analisis Penurunan Glukosa dari Sampel yang Disimpan Dalam Kaitannya dengan Pemisahan Serum dan Suhu Penyimpanan)

Gustamin, Liong Boy Kurniawan, Ruland DN Pakasi

ABSTRAK

Glukosa darah merupakan uji yang rutin untuk menilai bahaya penyakit diabetes melitus. Masalah utama yang dihadapi rumah sakit di daerah adalah sering terjadinya penundaan terhadap pemeriksaan glukosa darah, sehingga terjadi penurunan kadar glukosa akibat glikolisis. Penelitian ini bertujuan untuk mengetahui penurunan kadar glukosa darah berdasarkan suhu penyimpanan dan pemisahan serum. Penelitian ini merupakan penelitian observasional dengan pendekatan kohort kajian yang dilakukan di RS Dr. Wahidin Sudirohusodo masa waktu Agustus 2016. Kadar glukosa diukur dengan menggunakan ABX-Pentra 400. Data yang didapat dianalisis menggunakan uji statistik Shapiro wilk dan wilcoxon. Hasil penelitian didapat dari 22 sampel darah lengkap dengan perlakuan berbeda berdasarkan suhu penyimpanan dan pemisahan serum. Hasil menunjukkan perbedaan bermakna antara glukosa serum dengan beku yang disimpan selama 2 jam pada suhu ruang yang mengalami penurunan sebesar 9,32 mg/dL jika dibandingkan dengan penyimpanan selama 2 jam pada suhu freezer yaitu sebesar 0,8 mg/dL ($p=0,000$) dan jika dibandingkan dengan serum pada suhu ruang selama 2 jam sebesar 1,38 mg/dL ($p=0,000$). Sampel untuk pemeriksaan glukosa yang tertunda sebaiknya dilakukan pemisahan antara serum dan beku eritrosit agar tidak terjadi penurunan glukosa.

Kata kunci: Glukosa, suhu penyimpanan, pemisahan serum

ABSTRACT

Blood glucose is a routine test to assess the risk of diabetes mellitus. The major problem in the remote hospitals is the delay in processing blood glucose test sample resulting in a decreased glucose level due to glycolysis. This study aim was to determine the decrease in blood glucose levels based on the storage temperature and serum separation. This was a cohort study performed at the Dr. Wahidin Sudirohusodo Hospital in August 2016. Glucose levels were measured by using Pentra ABX-400. The data obtained were analyzed using Shapiro-Wilk statistical test and the Wilcoxon test. The study results were obtained from 22 whole blood samples treated differently based on storage temperature and serum separation. The result showed a significant difference between the unseparated serum samples stored at room temperature for 2 hours with a decrease of 9.32 mg/dL compared with the samples stored in the freezer for 2 hours with a decrease of 0.8 mg/dL ($p=0.000$) and compared to serum stored at room temperature for 2 hours with a decrease by 1.38 mg/dL ($p=0.000$) serum. Samples for glucose tests when pending should be separated from erythrocytes clots in order to avoid a decrease in glucose

Key word: Glucose, storage temperature, serum separation

INTRODUCTION

Glycolysis is the main route of glucose metabolism. Glycolysis is also considered as the main route for the metabolisms of fructose, galactose, and other

carbohydrates. The ability of glycolysis to produce ATP without oxygen is very important since it possibly occurs even though there is no oxygen.¹

Glucose undergoes phosphorylation and metabolism with lactate as the final product. This process requires

energy, adenosine triphosphate (ATP), which loses an inorganic phosphate, then converts it to adenosine diphosphate (ADP), and regenerates ATP, with the net result of two ATP molecules in each glucose molecule.^{1,2}

External glycolysis, moreover, occurs after blood samples are removed from the body. Serum or plasma glucose cooled at 0°C will be stable within 24 hours, while at room temperature, blood samples in the absence of glycolytic inhibitors will be metabolized, as a result, if blood samples are removed from the body in the absence of immediate examination, there will be a decrease.¹⁻⁵

Glucose, furthermore, can be used by erythrocytes to produce energy both inside and outside the body. Consequently, hemoglobin level in the blood can greatly affect the reduction of glucose levels stored at room temperature.^{2,5}

The main principle of blood glucose examination is that the examination should not be delayed. Nevertheless, there are some factors leading to a need for delays, such as a far distance from a place where samples are taken to a remote laboratory, as well as an emergency or urgent matter causing a delay in examining the samples. Delay in blood glucose examination usually occurs in local hospital laboratories due to the lack of availability of laboratory assessments and the far distance of the room where a patient is treated to the laboratory, resulting in delayed serum glucose examination.²⁻⁵

In normal red blood cell metabolism, glycolysis (breakdown of glucose) is the sole energy source for red blood cells. The red blood cells do not have mitochondria and depend entirely on glycolysis for energy needs. Glucose undergoes phosphorylation and then is metabolized with lactate as the final product.^{5,7}

This research aimed to determine the correlation of decreased glucose level to serum separation and storage temperature.

METHODS

This research was an observational study with a cohort study approach conducted during the period of August 2016 with ethical approval number (UH 16060617). Documentation was conducted after whole blood samples were examined. Firstly, whole blood samples were taken using red tubes and then divided into four tubes before centrifugation. Secondly, the first tube without erythrocyte clot was stored at room temperature (25-27°C), whereas the second

tube without erythrocyte clot was stored at freezer temperature (- 20°C). Thirdly, the third tube without serum separation was stored at room temperature, while the fourth tube without serum separation was stored at freezer temperature. Fourthly, each tube was examined at the second hour.

Next, data analysis was performed by using SPSS. The statistical method was used to calculate mean, Standard Deviation (SD) and data distribution. Afterwards, Shapiro-Wilk test and Wilcoxon test were carried out. Results of those statistical tests then could be considered to be significant if the value of p was less than 0.05.⁶

RESULTS AND DISCUSSION

This research was performed to examine the effects of storage time and serum separation using erythrocyte clot on the decrease of blood glucose (mg/dL) in the samples examined during the period of August 2016.

Table 1 showed the basic data of blood glucose levels, instantaneously examined and examined at the second time, as well as changes in blood glucose treated differently according to the storage temperature and the separation of the erythrocyte clot. The greatest decrease in the blood glucose levels was after 2 hours stored at room temperature in samples without serum separation, about 9.2 mg/dL. Similarly, a research conducted by Rodriguez⁸, showed that blood glucose levels decreased in samples without serum separation at room temperature, about 5-10 mg/dL due to the process of glycolysis in red blood cells.

Table 2 illustrated the comparison of the instantaneous glucose level and the glucose level after storage at freezer temperature for 2 hours with serum separation. Although the decreasing of the glucose level was statistically significant (p=0.000), it was only 0.68 mg/dL. Table 2 also demonstrated the comparison of the instantaneous glucose level and the glucose level after stored at freezer temperature for 2 hours without serum separation. The decrease of the glucose level was statistically significant (p=0.000). This condition may be due to freezer temperature at which glycolysis can be inhibited.

Table 3 depicted the comparison of the instantaneous glucose level and the glucose level after being stored at room temperature for 2 hours with serum separation. The decrease of the glucose level was statistically significant (p=0.000), about 1.38 mg/dL. Table 3 also indicated the comparison of the instantaneous glucose level and the glucose level after stored at room temperature for 2 hours without serum

Table 1. Data of instantaneous blood glucose and blood glucose 2 hours after the storage based on storage temperature and serum separation

Data	Mean ± SD	Median	Min-Max
Instantaneous glucose	96±10.3	94	(86.8-124.5)
Glucose stored in freezer for 2 hours			
With serum separation	95.02±10.4	93.1	(85.7-124.5)
Without serum separation	95.37±10.4	93.4	(85 – 124.5)
Glucose stored at room temperature for 2 hours			
With serum separation	95.97±10.4	93.4	(85-124.5)
Without serum separation	95.4±10.41	92.7	(85-123.8)
Decrease of glucose stored in freezer for 2 hours			
With serum separation	86.46±11.1	84	(72.3-116.3)
Without serum separation	0.68±0.33	0.73	(0.00-1.2)
Decrease of glucose stored at room temperature for 2 hours			
With serum separation	1.38±0.67	1.3	(0.33-3)
Without serum separation	9.2±5.04	8.4	(1.23-21.7)

Source: primary data

Table 2. Changes in blood glucose levels after storage at freezer temperature for 2 hours

Instantaneous glucose level (mg/dL)	Glucose level after storage with serum separation for 2 hours (mg/dL) (Mean ± SD)	Decrease of glucose level after stored with serum separation for 2 hours (mg/dL)	P
96±10.25	95.02±10.39	0.68±0.33	0.000*
Instantaneous glucose level (mg/dL)	Glucose level after storage without serum separation for 2 hours (mg/dL) (Mean ±SD)	Decrease of glucose level after stored without serum separation for 2 hours (mg/dL)	P
96±10.25	95.37±10.37	1.38±0.67	0.000*

* Non-parametric Wilcoxon test test

* p <0.05: significant

Table 3. Changes in blood glucose levels after storage at room temperature for 2 hours

Instantaneous glucose level (mg/dL) (Mean ± SD)	Glucose level after storage with serum separation for 2 hours (mg/dL) (Mean ± SD)	Decrease of glucose level after storage with serum separation for 2 hours (mg/dL) (Mean ± SD)	P
96±10.25	95.37 ± 10.37	1.38 ± 0.67	0.000*
Instantaneous glucose level (mg/dL) (Mean ± SD)	Glucose level after storage without serum separation for 2 hours (mg/dL) (Mean ±SD)	Decrease of glucose level after storage without serum separation for 2 hours (mg/dL) (Mean ±SD)	P
96 ± 10.25	86.46 ± 11.08	9.32 ± 5.04	0.000*

* Non-parametric test : Wilcoxon test

* p<0,05:significant

Table 4. The comparison of glucose level between the samples stored with serum separation and the samples stored without serum separation after storage for two hours at freezer temperature

Parameters	Freezer temperature (Mean ± SD)	P
Decrease of glucose storage in freezer for 2 hours with serum separation (mg/dL)	0.68±0.33	0.000*
Decrease of glucose storage in freezer for 2 hours with serum separation (mg/dL)	0.80±0.55	

* Non-parametric test: Wilcoxon test,

* p <0.05: significant

separation. The decrease of the glucose level was statistically significant ($p=0.000$), about 9.32 mg/dL.

Table 4 showed the comparison of the decrease in the glucose level after stored at freezer temperature for 2 hours with serum separation to that of the glucose level after stored at freezer temperature for 2 hours without serum separation. The decrease of the glucose level was statistically significant ($p=0.000$), but the mean difference was 0.12 mg/dl. This condition may be caused by the fact that the freezer temperature can inhibit glycolysis in erythrocytes.

Table 5 depicted the comparison of the decrease in glucose level after stored at room temperature for 2 hours with serum separation to that of the glucose level after stored at room temperature for 2 hours without serum separation. There was a statistically significant difference in the glucose levels stored at room temperature with serum separation and without serum separation ($p=0.000$) with the mean difference of 8.64 mg/dL.

Table 6 demonstrated the comparison of the decreasing of the glucose level after stored at room

temperature for 2 hours with serum separation to that of the glucose level after storage at freezer temperature for 2 hours with serum separation. There was a statistically significant difference in the glucose levels stored with serum separation between at room temperature and at freezer temperature ($p=0.000$) with the mean difference of 0.7 mg/dL.

Table 7 illustrated the comparison of the decrease of the glucose level after stored at room temperature for 2 hours without serum separation to that of the glucose level after stored at freezer temperature for 2 hours without serum separation. There was a statistically significant difference in the glucose levels stored without serum separation between at room temperature and at freezer temperature ($p=0.000$) with the mean difference of 8.52 mg/dL.

The results of this research, furthermore, indicated that the storage without serum separation at room temperature will trigger a significant decrease in the blood glucose level due to glycolysis in erythrocytes using glucose as an energy source. Glucose is the main source of energy for the metabolism of red blood cells

Table 5. The comparison of glucose level between the samples stored with serum separation and the samples stored without serum separation after storage at room temperature for two hours

Parameters	Room temperature (Mean \pm SD)	P
Decrease of glucose storage at room temperature for 2 hours with serum separation (mg/dL)	0.68 \pm 0.33	0.000*
Decrease of glucose storage at room temperature for 2 hours without serum separation (mg/dL)	9.32 \pm 5.04	

* Non-parametric test: Wilcoxon test

* $p < 0.05$: significant

Table 6. The difference of changes in glucose levels with serum separation between 2 hours after stored at freezer temperature and 2 hours after storage at room temperature

Parameters	Decrease of glucose stored in freezer for 2 hours with serum separation (mg/dL) (Mean \pm SD)	P
Freezer temperature	0.68 \pm 0.33	0.000*
Room temperature	1.38 \pm 0.67	

* Non-parametric test: Wilcoxon test

* $p < 0.05$: significant

Table 7. The difference of changes in glucose levels without serum separation between 2 hours after stored at freezer temperature and 2 hours after stored at room temperature

Parameters	Decrease of glucose stored in freezer for 2 hours without serum separation (mg/dL) (Mean \pm SD)	P
Freezer temperature	0.80 \pm 0.55	0.000*
Room temperature	9.32 \pm 5.04	

* Non-parametric test: Wilcoxon test

* $p < 0.05$: significant

through the pathway of glycolysis. In this process, one glucose molecule will give a net result of two ATP molecules. Glycolysis under normal circumstances will lower blood glucose levels in vitro by 5-10 mg/dL per hour at room temperature (25-27°C). Red blood cell metabolism stored at 2-6°C, on the other hand, undergoes a slowdown, allowing blood to survive during the storage. Besides, temperatures above 6°C allow bacterial activity.^{7,8}

Mature red blood cells, moreover, have no nuclei and mitochondria, so they cannot produce energy through the Krebs cycle (oxidative pathway). The red blood cells are completely dependent on other metabolic pathways, especially; Embden-Meyerhof pathway (glycolysis), in which ATP is produced by anaerobic glucose breakdown; The Hexose Monophosphate line, which produces NADPH to protect red blood cells from oxidative injury; The Rapoport-Luebering pathway, producing 2,3-diphosphoglycerate (2,3-DPG) which plays a role in the regulation of oxygen affinity.^{8,9}

In addition, ATP level usually will be steady or slightly increase during the early blood storage, then will reach it peak in two weeks, and eventually will decrease by 50% at the end of the blood storage. Next, in the early of glycolysis process, ATP is essentially needed, and even can make the glycolysis process stop if ATP reduces or runs off. ATP plays a role in maintaining the phospholipid membrane of red cells. Decreased ATP during storage, as a result, can lead to changes in red blood cells, from biconcave to spherocyte, decreased lipid content, as well as increased cell rigidity.^{5,8,9}

CONCLUSION AND SUGGESTION

In conclusion, there was a difference in glucose levels between the samples with serum separation and

those without serum separation at room temperature storage. There was also a difference in glucose levels of the samples stored at room temperature from those stored at freezer temperature, either with serum separation or without serum separation.

Thus, blood glucose examination should be performed immediately. As a result, there will be no decrease in glucose levels due to glycolysis process. Besides, delayed blood sample examination had better be performed by separating between serum and erythrocyte clots.

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