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Differences in Blood Glucose Levels of NaF Plasma with Blood Stored at Room Temperature

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Abstract. Blood glucose is sugar contained in the blood, derived from carbohydrates in the diet, and stored as glycogen in the liver and skeletal muscles. Blood glucose examinations are widely recommended by paraclinics, which aim to screen or monitor diabetes mellitus. Ideally, blood glucose levels should be checked immediately. This study aims to determine the difference in blood glucose levels in NaF plasma immediately checked with stored blood after 2 and 4 hours of storage at room temperature. This type of research is comparative analysis. The sample is venous blood from as many as 30 respondents, taken by random sampling. The blood glucose level in NaF plasma was immediately examined by the GOD-PAP method, and as a comparison, the blood glucose level in NaF plasma with blood stored for 2 hours and the blood glucose level in NaF plasma with blood stored for 4 hours Blood was put in three different NaF (Sodium Flouride) tubes: immediately examined blood, 2-hour stored blood, and 4-hour stored blood. The results of the study found differences in blood glucose immediately checked with blood stored for 2 hours and 4 hours at room temperature with a value of $p > 0.05$ ($p = 0.001$ and 0.002). The conclusion of this study was that significant differences were found between blood glucose levels immediately checked with blood stored for 2 hours and 4 hours at room temperature.

Keywords: Carbapenems, resistant, Gram-negative bacilli

1. Introduction

Clinical laboratory tests play an important role in disease diagnosis, including blood glucose tests, which are often used for screening and monitoring of diabetes mellitus(Fishel Bartal et al. 2023; Liu 2012). To obtain accurate results, there are several influencing factors, such as patient preparation, which includes fasting or non-fasting conditions, the sample collection process, sample preparation, and the examination method used. Blood glucose levels can be measured from whole blood samples, either from capillary or venous blood vessels, in the form of serum or plasma. The normal range of values for blood glucose levels using serum or plasma samples is usually between 70 and 110 mg/dL. Some types of anticoagulants used in blood glucose testing are sodium fluoride (NaF), sodium oxalate, sodium citrate, or lithium heparin.(Noor 2017).

Blood glucose is a form of sugar found in the circulating blood, produced from carbohydrates in the diet, and stored as glycogen in the liver and skeletal muscles. Its function is as a source of energy for the body and its tissues(Nisa 2020). Blood sugar is the amount of glucose in the blood plasma. Factors that can affect blood sugar levels include increased food intake, increased stress and emotional factors, increased weight and age, and exercise(Harymbawa and Aditya 2016).

Diabetes is a serious, non-communicable disease in which the pancreas is unable to produce insulin optimally. Insulin is the hormone responsible for regulating glucose levels in the body. When insulin is not functioning properly, blood glucose levels can increase. Normally, blood glucose levels during fasting range from 70 to 110 mg/dL(Fatimah 2015).

As a commonly used anticoagulant in blood sampling for glucose level measurement, sodium fluoride (NaF) is considered to have the ability to inhibit the glycolysis process. NaF can maintain

the stability of blood glucose levels for 24 hours at room temperature. In addition, NaF also has a precipitating effect on calcium ions (Ca^{++}), so it can prevent glycolysis by inhibiting the activity of the enolase enzyme(Gandasoebrata 2013).

Delays in glucose testing can cause a decrease in glucose levels which can be caused by red blood cells, white blood cells, platelets and bacterial contamination. Blood cells and bacteria maintain their life by utilizing glucose as an energy source in metabolism so that the process of glycolysis occurs(Nisa 2020). Blood glucose testing is often delayed due to several factors, such as waiting until the number of samples reaches the specified limit for efficient testing, equipment malfunction that requires the addition of anticoagulants to delay samples, shortage of staff, delayed transportation of samples to the laboratory, and lack of equipment and reagent facilities in village health centers. In these cases, village health centers with limited facilities need to refer the examination to places with more adequate facilities (Assyifa 2016).

2. Metode

2.1 Research Design

This study uses a comparative analytic method that aims to compare the presence of one or more variables, in two or more different samples, and at different times. This research was conducted at the Clinical Chemistry Laboratory of the Banten Ministry of Health Polytechnic in February 2022.

2.2 Population and Sample

This study involved a population of D-III Medical Laboratory Technology study program students at the Banten Ministry of Health Polytechnic campus. Samples in the form of venous blood were taken directly by researchers from the Clinical Chemistry Laboratory of the Banten Ministry of Health Polytechnic in February 2022. The sample size was 30 youth donors determined using the Slovin Formula based on a population of 105 students.

2.3 Analisis Data

Data analysis in this study involved pre-requisite analysis tests, namely normality test and homogeneity test, and continued with the analysis of significant differences using the One Way ANOVA test.

3. Results

Based on Table 1, it is known that the average blood glucose level in NaF plasma immediately examined is 107.85 mg/dL, while in 2-hour and 4-hour stored blood is 96.61 mg/dL and 86.29 mg/dL, respectively. The standard deviations for the three groups were 111.24; 113.42; and 114.88, respectively. The maximum value obtained was 124.7 mg/dL, while the minimum value was 62.7 mg/dL.

Table 2 shows that the results of the examination of blood glucose levels in NaF plasma show that the data in the immediately examined condition, 2-hour stored blood, and 4-hour stored blood have p values of 0.114, 0.546, and 0.769, respectively, all of which are greater than 0.05. This indicates that the data is normally distributed. Therefore, the normality test showed that the data in this study had a normal distribution, so it was continued with the data homogeneity test.

Based on table 3 shows that the homogeneity test shows the results of a significant value of p = 972 (> 0.05), thus the normality test of this research data is declared normally distributed and homogeneous.

Table 1. Distribution results and average blood glucose levels in NaF plasma

No. Sampel	Gender	Glucose Level(mg/dL)		
		Check immediately	Blood store 2 hours	Blood store 4 hours
1.	Female	94.7	81.2	75.3
2.	Male	120.1	111.9	102.3
3.	Female	110.0	79.0	65.8
4.	Female	116.4	97.1	86.2
5.	Female	118.9	112.3	100.9
6.	Female	102.8	98.2	89.9
7.	Female	119.2	101.2	89.9
8.	Female	114.6	100.0	94.0
9.	Female	104.7	97.8	89.0
10.	Female	110.5	100.8	90.5
11.	Female	120.0	113.4	101.2
12.	Female	98.5	89.0	79.5
13.	Female	99.2	91.7	75.2
14.	Female	111.5	99.6	79.9
15.	Female	97.6	85.1	79.0
16.	Female	93.3	83.9	78.9
17.	Female	98.2	92.1	82.2
18.	Female	109.4	77.2	62.7
19.	Male	120.8	108.0	95.0
20.	Female	87.6	83.7	76.8
21.	Male	124.7	119.6	109.1
22.	Female	120.5	110.3	97.1
23.	Female	111.7	102.2	93.6
24.	Female	119.6	99.8	85.0
25.	Female	99.2	92.0	83.7
26.	Female	112.1	101.9	96.6
27.	Female	102.2	97.6	88.1
28.	Female	116.2	104.4	96.8
29.	Female	99.4	90.3	80.1
30.	Female	81.9	77.1	64.6
Total		323.6	289.8	258.9
Mean		107.85	96.61	86.29
SD		111.24	113.42	114.88
Min		81.9	77.1	62.7
Max		124.7	119.6	109.1

Table 2. Data normality test

Variabel	Sig	Description
Check immediately	0,114	Normal
Blood store 2 hours	0,546	Normal
Blood store 4 hours	0,769	Normal

Sumber: Data Primer, 2022

Tabel 3. Uji Homogenitas Data

Tests of Homogenity of Variances	
Significant Results	p = 972

Tabel 4. Uji One Way ANOVA

Tests of One Way ANOVA		
Blood glucose levels		**Sig.**
Check immediately	Blood store 2 hours	.001
	Blood store 4 hours	.000
Blood store 2 hours	Check immediately	.001
	Blood store 4 hours	.002
Blood store 4 hours	Check immediately	.000
	Blood store 2 hours	.002

Table 4 shows a significant difference in the examination of blood glucose levels in NaF plasma. The results of the one-way ANOVA test showed significant differences between NaF plasma immediately examined with blood stored for 2 hours (p-value = 0.001 < 0.05), blood stored for 4 hours (p-value = 0.001 < 0.05), and between NaF plasma immediately examined with blood stored for 2 hours and 4 hours (p-value = 0.002 < 0.05). There was a significant difference between blood glucose levels in NaF plasma immediately examined and blood stored for 2 hours and 4 hours at room temperature.

4. Discussion

This examination uses the GOD-PAP reagent method from BIOLABO with a recipe of 1000 µl of reagent and 10 µl of control serum, with an incubation time of 10 minutes using a wavelength of 546 nm. The control material was control serum with LOT number 95011. The control range value is 177-240 mg/dL. On February 23, 2022 the control value was 232.0 mg/dL, on February 25, 2022 it was 180 mg/dL, and on February 28, 2022 it was 193.2 mg/dL.

Based on the examination of 30 samples, the results showed that the blood glucose level in NaF plasma examined directly had an average of 107.85 mg/dL, with the lowest value of 81.9 mg/dL and the highest value of 124.7 mg/dL. Meanwhile, after the blood was stored for 2 hours, the examination of blood glucose levels in NaF plasma resulted in an average of 96.61 mg/dL, with the lowest value of 77.1 mg/dL and the highest value of 119.6 mg/dL. Furthermore, after the blood was stored for 4 hours, the examination of blood glucose levels in NaF plasma resulted in an average of 86.29 mg/dL, with the lowest value of 62.7 mg/dL and the highest value of 109.1 mg/dL. It can be seen that there is a decrease in blood glucose levels by 10% from the initial value. So the results show that the immediate glucose level check will produce a higher result value when compared to the delayed stored blood.

In table 2, a normality test has been carried out to determine whether the data is normally distributed or not. The normality test is part of the statistical analysis requirements test. In the normality test, data is said to be normally distributed if it has a significant value of more than 0.05. The value of blood glucose levels in NaF plasma immediately examined was p = 0.114 (> 0.05), blood glucose levels in NaF plasma stored blood 2 hours by p = 0.546 (> 0.05), and blood glucose levels in NaF plasma stored blood 4 hours by p = 0.769 (> 0.05), so the data is normally distributed because it has a value of p> 0.05.

In table 3, a homogeneity test is carried out, this test is carried out to determine whether several population variants are the same or not. The results show a significant value of p = 0.972 (> 0.05) so it can be concluded that the data is normally distributed. In table 4 of the One Way ANOVA test, this test was conducted to determine whether or not there was a difference between the three treatment groups conducted by researchers on glucose levels obtained significant results in NaF plasma which was immediately checked against 2-hour stored blood with p value = 0.001 (<0.05),

immediately checked against 4-hour stored blood with p value = 0.001 (<0.05). Then, immediately examined with blood stored 2 hours and blood stored 4 hours p value = 0.002 (<0.05), the results showed a significant difference. So it can be concluded that there is a significant difference between blood glucose levels in NaF plasma immediately examined with blood stored for 2 hours and 4 hours at room temperature. This finding is consistent with the results of previous studies conducted by Agung et al. (2017) and Kasimo (2020) who also examined the effect of delayed testing on blood glucose levels.

Agung et al. (2017) found that at the delay time before 2 hours, 4 hours, and 8 hours, there was no significant difference between fresh serum, serum stored 1 hour, NaF plasma immediately, and NaF plasma stored 1 hour. This can be explained by the relatively short delay time, so the decrease in glucose concentration was not clinically significant.

In a study conducted by Kasimo (2020) showed a significant decrease in glucose levels after a 12-hour delay in examination in patients with diabetes mellitus. This shows that a longer examination delay can have a significant effect on the results of measuring glucose levels in NaF plasma.

This research is different from the research findings by Yuliandi & Lokananta (2022) which concluded that there was no significant difference between the time delay of 0-4 hours on blood glucose levels in NaF plasma. Therefore, the results of blood glucose examination on NaF plasma delayed for 0-4 hours can still be used. The possibility of differences in research results, according to Yuttana (2014) may be caused by various factors that have not been studied with certainty, such as differences in the number of samples used, variations in storage temperature, examination room conditions, and other factors. In addition, potential biases that can arise due to human or device error can affect the results by giving values that are too low or too high.

There was a difference in NaF plasma glucose levels between the immediate measurement and the delayed measurement for 2 hours and 4 hours. The decrease in glucose levels observed in the 2-hour and 4-hour delays may be caused by the process of glycolysis, especially when the glucose test is delayed. Delayed testing can occur due to obstacles in the testing process. One of the methods used to delay blood glucose testing is by using a sodium flouride anticoagulant. NaF anticoagulant functions as an antiglycolytic substance that can prevent glucose metabolism, thus maintaining the stability of glucose levels in the blood(Nugraha 2015). The results showed that the blood thinner NaF, which blocks glycolysis, could not stop the drop in glucose levels after two and four hours.Evidence was seen from the decrease in glucose levels in NaF plasma samples that had been delayed for 2 hours and 4 hours.

5. Conclusion

The research showed that there was a difference in the amount of glucose in the blood when NaF plasma was tested right away compared to blood that had been stored at room temperature for 2 hours and 4 hours.Statistical analysis showed that the difference had a high level of significance, with a p value of <0.05. The results also showed a downward trend in glucose levels in NaF plasma as blood storage time increased. This indicates that blood storage time can affect the measurement results of glucose levels in NaF plasma.

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